

ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE
Fiscal Year 1980
Part VI-B

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

ANNUAL REPORT

OF

PROGRAM ACTIVITIES

United States NATIONAL CANCER INSTITUTE

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Part VI-B

Division of Cancer Treatment

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR CLINICAL ONCOLOGY PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1979 - September 30, 1980

The Clinical Oncology Program consists of seven Branches: the Medicine Branch, Radiation Oncology Branch, Pediatric Oncology Branch, Surgery Branch, Biometric Research Branch, and Clinical Pharmacology Branches are located in the Clinical Center in Bethesda; the NCI-VA Medical Oncology Branch operates at the Washington Veterans Administration Hospital. These branches conduct and support clinical treatment trials in patients with a variety of neoplasms. The major treatment thrusts are Phase I, II, and III clinical therapeutic studies in those tumors which represent the best clinical models to study the question at hand. In recent years, combined modality treatment has been strongly emphasized, particularly the use of adjuvant chemotherapy and radiation therapy, and the use of intensive hematologic and microbiologic support. The program is also involved in the Phase I-II testing of new anti-cancer drugs, and in the study of pharmacokinetics of these agents. Finally, each Branch conducts laboratory research in topics related to clinical investigation. These include basic studies in clinical pharmacology, cell biology, cytogenetics, tumor cell kinetics, tumor immunology, biological cell modifiers, various aspects of cancer etiology, epidemiology, and toxicology. Details of the progress of individual Branches are listed in the annual report under the specific Branch headings.

Program Accomplishments

Office of the Associate Director for Clinical Oncology/Deputy Clinical Director

This office is responsible for program planning, coordination, and administration of the intramural Branches. In addition, all clinical activities of the NCI, including patient recruitment, protocol review, patient care, bed allocation, and the postgraduate oncology training programs are the responsibility of the Deputy Clinical Director.

Several projects have been developed by this office.

1. Chemoprevention of Cancer

The Associate Director serves as Chairman of an interdivisional working group on the chemoprevention of cancer. Several workshops have been held on this topic, and two clinical trials, prevention of skin cancer in albino Africans, and prevention of cervical cancer in women with cervical dysplasia, were initiated.

2. Study of Bilharzial Bladder Cancer in Egypt

This study, now in its third year, tests preoperative radiation therapy as an adjunct to cystectomy for this disease. Preliminary results indicate a survival advantage in irradiated patients. This study also conducts phase II studies in patients with advanced bladder cancer. A current protocol compares hexamethylmelamine with its analogue, pentamethylmelamine. Misonidazole is also in phase II testing as a radiation sensitizer.

3. Effect of Chemotherapy on Reproductive Capacity

A working group, chaired by the Associate Director, meets regularly for collaborative studies on this topic. It has been shown that more than half the surviving women receiving chemotherapy develop amenorrhea and/or early menopause. Women who do receive anticancer drugs, exclusive of the first trimester of pregnancy, may be able to deliver normal children. Men receiving chemotherapy have a high incidence of azospermia which may be irreversible.

4. Revision of Oncology Training Program

A new plan to begin in July 1982, allows medical oncology fellows to elect a one year program or two six month rotations (NCI-Navy, POB, MB) for the first year of a guaranteed 3-year training program. Candidates will be accepted into the overall clinical oncology program, rather than to specific Branches as in the past.

Other major areas of progress in the Clinical Oncology Program in the past year are summarized below, and specific details are documented in the individual Branch Annual Reports.

1. The intracellular degradation of methotrexate into polyglutamates is accomplished by an enzyme, DCMP deaminase, which controls de novo synthesis of DCPT. Regulation of this enzyme by nucleotide inhibitors and activators has been thoroughly characterized.
2. Metabolism of 5-FU to FdUMP and enhanced incorporation into RNA has been demonstrated in the presence of new pyrimidine antagonist, PALA. This important drug interaction is now being tested in a clinical trial which 3 of 9 patients thus far evaluable have achieved tumor regressions.
3. Melatonin suppresses the development of breast cancer in rats exposed to the carcinogen DMBA. This suppression is correlated with suppression of prolactin secretion by the pituitary gland and suggests that melatonin exerts important influence on prolactin secretion in animals.
4. In vitro studies of combinations of hydroxyurea and adriamycin, or cytosine arabinoside, have been performed and show synergistic activities between these drugs.

5. Continued studies of the administration chemotherapeutic agents by the intraperitoneal route have been carried out. The pharmacokinetics of intraperitoneal methotrexate, 5-FU, and adriamycin, have been characterized. All of these studies indicate that high levels of these drugs can be achieved in the peritoneum without systemic toxicity.
6. It has been demonstrated in ovarian cancer that a new combination called CHEXUP (cyclophosphamide, hexamethylmelamine, 5-FU and cis-platinum) produces a complete remission rate of 52% in patients with ovarian cancer, an improvement over a 33% rate from a previous drug combination.
7. Extensive experience has accumulated with the human tumor stem cell assay. Over 60 patients with ovarian cancer have been studied, and the clinical correlation in determining drug sensitivity by this assay exceeds 90% for inactive and 64% for active agents.
8. A ten-year followup of 98 patients with advanced Hodgkin's disease has been completed. Complete remission rate is 80%. Sixty-eight per cent of the complete responders achieved continuous disease-free survival. Overall survival for those achieving remission is 82%, indicating a substantial "salvage" rate for patients who relapse.
9. A persistent defect in T-cell function has been discovered in Hodgkin's disease patients who have been cured. This defect appears to be intrinsic to the host and is not a consequence of the therapy or the presence of active disease.
10. A complete analysis of 473 patients with non-Hodgkin's lymphoma has been undertaken. Among the many publications resulting from this analysis is the important observation of histologic conversion from nodular to diffuse lymphoma. This has led to the development of a prospective trial comparing two treatment strategies: "watch and wait"- a conservative approach vs. intensive therapy aimed at cure.
11. A prospective study in breast cancer, in which tamoxifen treatment with or without halotestin was studied, suggests that combination is superior to tamoxifen alone. Another randomized trial shows that estrogen receptor positive patients may benefit from hormone synchronization in conjunction with chemotherapy.
12. A large trial of malignant melanoma has been completed recently. This trial revealed no substantial relapse or survival advantages amongst patient groups who received methyl-CCNU, BCG or BCG plus tumor cell vaccine as adjuvant treatments.
13. A major study on small cell bronchogenic carcinoma has revealed that chemotherapy plus radiation therapy yields superior results to either modality alone in patients with limited disease extent.
14. In patients with extensive small cell carcinoma, combination chemotherapy has established a 63% overall response rate and a 36% complete response rate.

15. Phase II trials of systemic hyperthermia in combination with cancer chemotherapeutic agents has revealed no distinct advantage conferred by hyperthermia.
16. Study of radiation therapy plus misonidazole in esophageal cancer is underway. Thus far, eight patients have been treated, three of whom have had no evidence of tumor after thoracotomy.
17. A study of intraoperative chemotherapy for retroperitoneal sarcoma, gastric carcinoma and pancreatic cancer has begun. Thus far, seven patients have been treated.
18. A direct comparison of surgery versus radiotherapy for the treatment of breast cancer is underway, with 25 patients entered in the trial. Preliminary results reveal equivalent effects of both treatments, indicating radiation alone as a potential, breast-saving, treatment modality in this disease.
19. A three-fold survival improvement has been achieved in patients with osteosarcoma with combined modality treatment. The 4-year survival is 68%, as compared to 18% in historical controls.
20. A doubling of disease-free overall survival in patients with soft tissue sarcoma with combined modality treatment has been demonstrated. Limb-sparing treatment with survival equivalent to amputation can be achieved with patients with sarcomas of the extremity.
21. The value of total parenteral nutrition in improving the outlook of advanced cancer patients is being evaluated. Thus far no effect in treatment response or survival can be demonstrated, although tolerance to drug treatment is improved.
22. Surgical debulking in testicular cancer plus combination chemotherapy has substantially increased the complete response rate and survival in these patients.
23. Collaborative work with the Radiology Department has resulted in a refinement in the detection of liver metastases using emulsified lipiodol preparation with the CAT scan.
24. Long-term growth and expansion of cytotoxic T cells has been accomplished through the use of T-cell growth factor and cloning techniques. These clones are under evaluation as effector cells in adoptive immunotherapy of cancer.
25. Forty young patients with non-Hodgkin's lymphoma were treated with cytoxan, adriamycin, oncovin, and prednisone, alternating with high-dose 42-hour intravenous methotrexate infusions. Seventy percent of these 40 patients are alive and disease-free for more than 6 months. Most relapses were isolated in the CNS, indicating that if successful CNS prophylaxis can be developed, durable remissions in about 80% are achievable.

26. Patients with Ewing's sarcoma have been treated with intensive combination chemotherapy and total body irradiation, plus bone marrow rescue in the form of autologous bone marrow infusions. Remission rate, remission duration, and survival are significantly better than has been the case with any previous NCI treatment protocol for metastatic Ewing's sarcoma.
27. The somatic cell hybrid system has been used to demonstrate that the pattern of expression of the human alpha-globin genes depends on the differentiated state of the donor cell. Studies of the expression of myeloid differentiation, using the human leukemia cell line, HL60, show that the morphologic changes mimic normal myeloid differentiation. Specifically, HL60 cells can be induced to acquire those attributes which are necessary for the integrated function of chemotaxis.
28. Collaborating with the Metabolism Branch, it was shown that patients with infectious mononucleosis initially have increased numbers of circulating cells capable of secreting immunoglobulin, whereas later there is a striking reduction in these cells. This observation strongly suggests that initial B-cell activation is brought about in vivo by Epstein-Barr virus infection, but that this activation is subsequently controlled by the generation of powerful suppressor T-cells.
29. The outcome of pregnancies of patients who receive aggressive chemotherapy was examined. Of 448 patients screened, 30 had 12 abortions (10 elective and 2 spontaneous) and 29 live births. Followup of offspring revealed no major malformations. In addition, growth, development and school performance were normal. These results support the contention that chemotherapy administered to women prior to gestation or after the first trimester may result in normal births in the majority of cases.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06308-09 BR
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PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Biometric Research Branch

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Simon	Chief, Biometric Research Branch	COP, DCT, NCI
OTHER:	B. K. Edwards	Expert	COP, DCT, NCI
	R. W. Makuch	Senior Investigator	COP, DCT, NCI
	M. N. Wesley	Staff Fellow	COP, DCT, NCI
	R. A. Wesley	Expert	COP, DCT, NCI

COOPERATING UNITS (if any) Clinical Oncology Program, DCT, NCI; Developmental Therapeutics Program, DCT, NCI; Cancer Therapy Evaluation Program, DCT, NCI; Baltimore Cancer Research Program, DCT, NCI; Immunology Branch, DCBD, NCI; Laboratory of Pathology, DCBD, NCI; Dr. D. Von Hoff, University of Texas.

LAB/BRANCH

Biometric Research Branch

SECTION:

INSTITUTE AND LOCATION

NCI, National Institutes of Health, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

5.75

PROFESSIONAL:

5.0

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☒ (c) NEITHER

☐ (a1) MICROS

☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The branch is the statistical component of the Division of Cancer Treatment. The branch designs, conducts and analyzes intramural and national clinical trials of experimental treatments, and conducts studies to identify important prognostic and treatment selection factors. Collaborations with the Developmental Therapeutics Program are directed to evaluating and improving methodology for pre-clinical drug screening and synthesis. The branch collaborates with the Cancer Therapy Evaluation Program in the review and coordination of national clinical therapeutic research in oncology. The branch designs and maintains computerized data collection systems and develops new biometric methods. A list of current studies follows.

1. Design and Conduct of Intramural Clinical Trials

A member of the BRB serves as statistician for all major clinical trials conducted by the Clinical Oncology Program (COP) or the Baltimore Cancer Research Program (BCRP). This activity includes protocol development, randomization, data collection and analysis. New clinical trials in which the branch collaborates include the following. (1) Randomized evaluations of intra-operative radiotherapy with misonidazole for the treatment of pancreatic cancer, gastric cancer, and retroperitoneal soft tissue sarcomas. (2) Randomized evaluation of intensive high dose methotrexate for the adjuvant treatment of osteosarcoma. (3) Proposed randomized evaluation of lymphadenectomy for stage I non-seminomatous testicular cancer. (4) Randomized evaluation of excisional biopsy with axillary dissection and irradiation for the treatment of stages I-II carcinoma of the breast. (5) Randomized evaluation of nerve cutting procedures on the development of phantom pain for patients receiving amputations. In addition to these new studies and active involvement in ongoing studies, a member of the branch serves on the Clinical Research Panel and on the Data Monitoring Committee to review all intramural clinical trials.

2. Extramural Clinical Studies

2.1 Head and Neck Cancer

The branch serves as statistical center for the national studies of head and neck cancer. The studies are conducted by 13 contract institutions and two cooperative groups. The studies are randomized evaluations of pre-operative chemotherapy and of maintenance chemotherapy following surgery and radiotherapy. Approximately 250 patients have been registered out of a required target of 600. Data quality, therapeutic consistency and toxicity are closely monitored.

2.2 Stages I-II Ovarian Cancer

The branch serves as statistical center for national studies of early ovarian cancer conducted by the Gynecologic Oncology Group and Ovarian Cancer Study Group. The studies are randomized evaluations of adjuvant chemotherapy or implanted isotope radiotherapy. The usefulness of clinical and surgical staging procedures is also evaluated. Patient accrual is continuing.

2.3 Diet, Nutrition and Cancer Program (DNCP)

A member of the branch has served as chief statistician for clinical studies of the DNCP. These studies include the following three areas.

- (a) Randomized evaluations of total parenteral nutrition (TPN) as an adjunct to cancer therapy in pediatric patients. These studies included patients with abdominal malignancies treated with radiotherapy, and those with metastatic bone involvement treated with high dose methotrexate.
- (b) Observational investigation of the relationships among nutritional status, disease symptoms, and disease course for pediatric patients with cancer.
- (c) Randomized evaluations of TPN as an adjunct to cancer therapy in the adult. Separate studies were performed for head and neck cancer, non oat cell lung cancer, testicular cancer and advanced colon cancer.

Within the past year final analyses have been performed by the chief statistician for these studies and manuscripts are being submitted for publication. The branch has also assisted in transition of the DNCP data management operations to a new contractor.

2.4 Histological Classification of Non-Hodgkins Lymphomas

The branch has collaborated with the Laboratory of Pathology and the Radiation Oncology Branch to conduct an international study of histological classification systems. 1175 cases of carefully staged "modernly" treated lymphomas have been classified in the six major systems by a panel of expert pathologists. The analysis of the histological systems is completed and publications are being prepared. The panel of experts have agreed to recommend a uniform working classification system. The BRB will further analyze this data to address staging and therapeutic questions.

2.5 Osteosarcoma Working Group

The BRB will serve as statistical center for joint therapeutic studies in osteosarcoma to be performed by the NCI Surgery Branch, NCI Pediatric Oncology Branch and four extramural research institutions. One primary and one metastatic disease protocol are being finalized, and data management systems developed.

2.6 Metastatic Breast Cancer Working Group

The BRB has served as statistical center for three institutions performing joint therapeutic studies in metastatic breast cancer under contract to the NCI. During this year, the final study in this series, a randomized evaluation of four combination chemotherapy regimens, has been analyzed and submitted for publication. This collaboration has terminated.

2.7 Other Projects

(a) The BRB has done preliminary planning for its potential role as statistical center for joint studies in the treatment of pediatric cancers conducted by the NCI Pediatric Oncology Branch, Stanford University Medical School and the major military hospitals. Similar planning is being conducted for a national chemo-prevention study of retinoids for cervical dysplasia.

(b) The BRB works closely with the Cancer Therapy Evaluation Program to review the design of all major therapeutic studies funded by the NCI. A member of the branch also serves on the Clinical Oncology Review Committee to review the progress of contract funded clinical studies.

(c) The branch has participated in the statistical design of a national study to evaluate the effect of chemotherapy on pregnancy outcomes, and an Egyptian study for the evaluation of hexamethylmelamine and pentamethylmelamine in bladder cancer.

(d) Members of the branch have participated in the selection of statistical and coordinating centers for the Brain Tumor Study Group, Advanced Endometrial Cancer Study Group and for a national study of the long term effects of chemotherapy. Site visits were made of the statistical centers for two cooperative groups and source selection of a monitor for extramural phase 1-2 clinical trials was participated in. The branch participated on

the USA-USSR Working Groups for Lung Cancer and Breast Cancer in Moscow, on the program committee of the American Society of Clinical Oncology, on the development of WHO/EORTC/NCI guidelines for standardizing the reporting of clinical trial results, on FDA advisory committee for developing guidelines for the study of oncologic drugs, and on the development of NCI guidelines for the support of studies contributing to new drug applications.

3. Statistical Analyses

The branch conducts statistical analyses of the results of clinical and laboratory studies. During the past year these studies have included the following.

3.1 Therapeutic Clinical Studies

(1) Evaluation of hematologic recovery of Ewing's sarcoma patients following bone marrow infusion. (2) Evaluation of total parenteral nutrition in the treatment of patients with diffuse lymphomas. (3) Evaluation of ProMACE-MOPP combination chemotherapy for the treatment of diffuse lymphomas. (4) Analysis of physiologic response and toxicity in patients undergoing whole body hyperthermia. (5) Evaluation of transdermal scopolamine as an anti-emetic for patients receiving cis-platinum chemotherapy. (6) Evaluation of empiric antibiotic therapy with or without amphotericin for neutropenic cancer patients with FUO. (7) Evaluation of prophylactic bactrim plus erythromycin for cancer patients receiving intensive chemotherapy. (8) Evaluation of intra-lesional BCG for the treatment of deep stage I melanoma. (9) Evaluation of Methyl-CCNU or BCG or BCG+neuraminidase treated cells for the adjuvant therapy of stage II melanoma. (10) Evaluation of thymosin for the treatment of small cell lung cancer. (11) Evaluation of two methotrexate regimens versus BCG plus cells for the maintenance of remissions in ALL. Also, comparison of intrathecal methotrexate versus intrathecal ARA-C for CNS prophylaxis. (12) Evaluation of asparaginase-VDP chemotherapy for the treatment of ALL patients after failure of initial therapy. (13) Evaluation of very intensive chemotherapy for pediatric lymphoma patients. (14) Evaluation of tamoxifen and fluorymesterone for the treatment of metastatic breast cancer. (15) Evaluation of effectiveness of double blind design for clinical trials of THC. (16) Evaluation of prophylactic brain irradiation in patients with small cell carcinoma of the lung. (17) Evaluation of limited versus thorough prophylaxis in preventing periodontitis in patients receiving chemotherapy.

In addition to the above completed studies, the branch is involved in numerous ongoing clinical trials for which interim analyses are performed.

3.2 Clinical Studies of Prognostic Factors and Tumor Biology

(1) Evaluation of the frequency and prognostic significance of histological progression and discordance in patients with non-Hodgkin's lymphomas. (2) Comparison of histological classification systems for non-Hodgkin's lymphomas. (3) Evaluation of clinical features and prognosis for patients with nodular histiocytic lymphoma. (4) Evaluation of clinical, laboratory and histological prognostic factors for patients with diffuse lymphomas. (5) Analysis of estrogen receptor heterogeneity among breast cancer lesions of the same patient as an explanation of non-uniform response to hormonal

therapy. (6) Evaluation of whether there is an anti-leukemic effect of graft versus host disease. (7) Evaluation of tumor sites, stage, performance status, laboratory values and histological subtypes as prognostic factors for patients with small cell lung cancer. (8) Evaluation of the association between smoking status and prognosis for patients with small cell lung cancer. (9) Evaluation of diagnostic procedures for the detection of bone involvement in patients with malignant melanoma. (10) Evaluation of sources of variability in the histological classification of pediatric lymphomas. (11) Evaluation of the effect of smoking on serum glycoprotein levels. (12) Evaluation of the effect of measles infection on serum glycoprotein and cellularity. (13) Evaluation of scintogram, ultrasound and CAT scan for the diagnosis of liver metastases. (14) Evaluation of prognostic factors on the survival of sarcoma patients with lung metastases. Factors studied included bilaterality, number of nodules, time from diagnosis till metastatic disease, tumor doubling time and histology. (15) Analysis of published literature on prognosis of sarcoma patients following thoracotomy. (16) Analysis of prognosis of ALL patients following initial relapse. (17) Evaluation of the effect of thymosin on leukocyte migration inhibition in patients with head and neck squamous carcinoma.

3.3 Laboratory and Pre-Clinical Studies

(A) Clonogenic Assay

The BRB is deeply involved in various aspects of the clonogenic assay for human tumor cells. We have designed and are analyzing experiments to identify combinations of nutrients that maximally stimulate growth. We have evaluated the association between assay results and clinical response to chemotherapy for 123 patients studied at the University of Texas. Detailed analyses of the cloning of neuroblastoma cells and of lung cancer cells from bronchoscopy washings have been made. We are collaborating with Dr. Von Hoff in the ongoing conduct of his studies and are participating in the design of a prospective SWOG evaluation of the assay for selecting treatment for individual patients. We have collaborated with the Drug Evaluation Branch in the development and initiation of a national evaluation of the clonogenic assay for screening new anti-cancer compounds. The development of this design and RFP was a major undertaking. A member of the branch will serve as co-project officer and statistician for this study.

(B) Other Drug Screening Projects

The BRB interacts with the Drug Evaluation Branch on a variety of statistical issues related to drug screening.

(1) We have demonstrated improved reproducibility for CD8F1 mammary screen by standardizing initial tumor size and employing mixtures of four breis. These changes were incorporated in a new screening protocol.

(2) Analyzed comparative data for 112 compounds tested in subcutaneous (SC) and subrenal capsule (SRC) xenografts. We determined that for purposes of classifying the activity of these drugs, the SRC and SC screens are very similar. In view of the reduced costs of the SRC method, the SC xenograft screens were eliminated. We designed further experiments to identify major sources of variability limiting the reproducibility of the xenograft screens.

(3) Conducted an analysis of correlations between results in experimental animal screening models and clinical activity of 45 thoroughly tested drugs. Independent contributions of each model were evaluated.

(4) Developed a powerful statistical method for evaluating adriamycin analogs based on dose-survival experiments in mice. Confidence intervals for optimal T/C values and the ratio of toxic to optimal doses were determined.

(C) Other Laboratory Investigations

(1) Analysis of chemosensitivity of malignant cell lines based on DNA cross linking index. (2) Evaluation of the effect of melatonin and pinealectomy on tumor induction in rats. (3) Statistical design of EPA toxicology protocol. (4) Evaluation of risk factors for GVH in mice. (5) Evaluation of the effect of dose schedule of adriamycin on cardiotoxicity in mice. (6) Evaluation of the comparative cardiotoxicity of four anthracyclines. (7) Evaluation of BCG immunotherapy in the surgical adjuvant treatment of carcinogen induced rat colon tumors. (8) Evaluation of joint dose-response relationships for mixed leukocyte reactions with more than one stimulator population.

4. Clinical Data Base System Development

The branch has completed the first phase of the development, testing and implementation of a generalized file management system for clinical trials. This system will be used for all COP clinical trials, for extramural trials in which the BRB is statistical center, and will be made available to other statistical organizations. Extensive effort is currently devoted to developing the data forms for each type of cancer and initiating the data collection process for all COP branches.

5. Methodological Research

(1) Introduction and evaluation of composite randomization designs for clinical trials. Community physicians are randomized rather than their individual patients.

(2) Evaluation of common-treatment-group designs for clinical trials where each institution adopts only a subset of all treatments.

(3) Development of clinical trial designs with early termination when initial results do not appear promising for the new treatment.

(4) Development of simple methods for calculating confidence interval for a percentile (e.g. median) of a survival distribution.

(5) Evaluation of the validity of the generalized Wilcoxon test with unequal censoring.

(6) Study of methods for deciding how to treat an individual patient based on clinical trial results for heterogeneous populations.

(7) Study of the relevance of prognostic factors in planning the size of a clinical trial.

- (8) Determination of realistic sample sizes for surgical adjuvant studies.
- (9) Evaluation of statistical designs and methods of analysis for Phase II clinical trials.
- (10) Study of validity and power of analysis methods for adaptive stratification designs.
- (11) Evaluation of the effects of pre-stratification, post-stratification and over-stratification on the validity and power of the log-rank test.
- (12) Evaluation of the conditional power of the Fisher-Irwin test given early results.
- (13) Development of a classification monitoring system for protocol deviations and protocol problems in multi-institutional clinical trials.
- (14) Representation of survival curves for two or more treatment groups adjusted for a comparable mix of patients.
- (15) Evaluation of factors causing "worsening survival curve syndrome" with accumulating data.
- (16) Development of optimal designs for clonogenic assay screening with several drugs and limited tumor.
- (17) Development of the asymptotic distribution of test statistics for nonparametric two-way analysis of variance with incomplete unbalanced blocks; applications to drug induced DNA cross-linking experiments.
- (18) The design and analysis of studies evaluating exposure effect on reproductive outcomes.
- (19) Study of sample re-use methods for estimating error rates of classification rules, deriving confidence limits in nonlinear regression models, identifying prognostic factors in follow-up studies, and estimating relative risk in case control studies.
- (20) Study of the statistical basis for case control studies. Development of simple principles for analysis of such studies.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06402-10 CP		
PERIOD COVERED October 1, 1979 to September 30, 1980				
TITLE OF PROJECT (80 characters or less) Tumor Growth Kinetics and Chemotherapy				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 45%; vertical-align: top;"> PI: Stanley E. Shackney, M.D. Paul S. Ritch, M.D. Richard F. Geist, M.D. Shan-shan Chen, M.D. Amy Newberger, M.D. Robert Glazer, Ph.D. </td> <td style="width: 55%; vertical-align: top;"> Senior Investigator, CPB, DCT, NCI Clinical Associate, CPB, DCT, NCI Clinical Associate, CPB, DCT, NCI Visiting Fellow, CPB, DCT, NCI IPA, Medicine Branch, DCT, NCI LMCB, DTP, DCT, NCI </td> </tr> </table>			PI: Stanley E. Shackney, M.D. Paul S. Ritch, M.D. Richard F. Geist, M.D. Shan-shan Chen, M.D. Amy Newberger, M.D. Robert Glazer, Ph.D.	Senior Investigator, CPB, DCT, NCI Clinical Associate, CPB, DCT, NCI Clinical Associate, CPB, DCT, NCI Visiting Fellow, CPB, DCT, NCI IPA, Medicine Branch, DCT, NCI LMCB, DTP, DCT, NCI
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COOPERATING UNITS (if any) NCI-VA Medical Oncology Branch, DCT, NCI, Laboratory of Medicinal Chemistry and Biology, DTP, DCT, NCI, Medicine Branch, COP, DCT, NCI, Western Lymphoma Group				
LAB/BRANCH Clinical Pharmacology				
SECTION Office of the Chief				
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205				
TOTAL MANYEARS: 4	PROFESSIONAL: 2	OTHER: 2		
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords) <u>Computer modeling</u> studies of drug-induced lethal and sublethal damage have been carried out. A kinetic compartment model has been developed for the <u>Sezary syndrome</u>. In vitro studies of combinations of <u>hydroxyurea</u> and <u>adriamycin</u>, and <u>cytosine arabinoside</u> and <u>adriamycin</u> were performed. The lethality of <u>vincristine</u> in sarcoma 180 in vitro was studied. Flow cytometry studies are being carried out in <u>human lymphoma</u>, <u>melanoma</u>, <u>acute leukemia</u>, and various other <u>solid tumors</u> in man.				

1. Theoretical Studies

1.1. Computer Modeling Studies

1.1.1. The mammalian cell population kinematic simulator (MACKS)

A program for the simulation of drug-induced growth retardation of sublethally damaged cells has been incorporated into our comprehensive model for tumor growth. A multicompartiment pharmacokinetic simulation model has also been incorporated into the model, permitting the simulated administration of drug in vivo or in vitro, extracellular compartment distribution and excretion (in vivo), intracellular drug distribution, and extracellular drug activation and inactivation (in vivo or in vitro).

Preliminary simulations of pulse dose hydroxyurea effects suggest that (a) the postperturbation effects of hydroxyurea on the DNA histogram are most consistent with the recruitment of minimally affected slowly proliferating cells; and (b) the effects in late stages of growth are much less than in early stages of growth, raising serious theoretical questions regarding the usefulness of cell synchronization in clinical treatment regimens.

2. Experimental Studies

2.1. Drug Studies in Sarcoma 180 In Vitro

2.1.1. Adriamycin CxT studies

A paper describing the CxT effects of adriamycin in sarcoma 180 has been submitted for publication. The lethality of combinations of hydroxyurea, adriamycin, and ara-C are studied.

2.1.2. Adriamycin combination studies

Schedule-dependent synergism between hydroxyurea followed by adriamycin and ara-C followed by adriamycin was maximal when the interval between drugs was two hours. There was no synergism when the HU and adriamycin or ara-C and adriamycin were given simultaneously or when adriamycin preceded HU or ara-C.

2.1.3. Sangivamycin studies

Studies of sangivamycin in sarcoma 180 in vitro have shown that prolonged exposure to drug is required to achieve significant lethal effects, but that there is a steep dose response during prolonged drug exposure. ^3H TdR and ^3H U uptake studies have demonstrated a delayed and dose-dependent inhibition of both DNA synthesis and RNA synthesis.

2.1.4. Vincristine studies

CxT studies of the effects of vincristine in sarcoma 180 were carried out this year. The effects of VCR were found to be both time and drug concentration dependent. Surprisingly, brief exposure (1 hr) to high concentrations of drug were capable of producing large cell kills. Serial flow cytometry studies following drug washout indicated that there was a transient inhibition of cells in mitosis 4-8 hours after brief exposure to high drug concentrations, and that cell fragmentation occurred following release from mitotic arrest. In contrast during and after prolonged drug exposure, cytokinesis is inhibited and cells do not fragment but go on to become polyploid.

2.2. Experimental Mouse Bone Marrow Studies In Vivo

Experimental data on the effects of hydroxyurea in mouse bone marrow were entered into the computer and analyzed this year. Quantitative analysis confirmed that the central region of the marrow participated freely in the post-drug recovery process and repopulated itself sooner than the peripheral zones.

2.3. Artifacts of Mithramycin Staining in Flow Cytometry Studies

The fixation time-dependent hyperchromatism of mithramycin has been a problem in the clinical detection of aneuploidy by flow cytometry. This year we found that exposure of samples in ethanol at 37°C for two hours produces maximum hyperchromatism in both fresh and aged samples, but does not alter the relative position of aneuploid peaks. This is a major advance in flow-based clinical detection of aneuploidy.

3. Clinical Studies

3.1. Flow Cytometry - Clinical Studies

3.1.1. Non-Hodgkin's lymphoma - collaborative study with western lymphoma group

A manuscript describing our results in the first 61 patients has been submitted for publication. Ploidy studies have been carried out in 50 additional cases of lymphoma. Results are consistent with findings in our earlier series. Three of five large B-cell lymphomas were aneuploid, 2/10 small B-cell lymphomas, 0/14 B-cell CLL's, 3/10 T-cell lymphomas, and 0/7 reactive nodes were aneuploid. Several miscellaneous cases of hairy cell leukemia, ALL, and myeloma were diploid.

3.1.2. Non-Hodgkin's lymphoma - collaborative study with the Medicine Branch, DCT

Approximately 30 cases of lymphoma have been accrued in a study comparing flow data with radioautographic studies. The radiographs are in various stages of processing. This study will continue into the coming year.

3.1.3. Melanoma - collaborative study with the Medicine Branch, DCT

Dr. Amy Newburger, in collaboration with our group, carried out serial flow cytometry studies in 10 patients with multiple melanoma nodules with methotrexate and/or metryl CCNU. Responsive patients showed persistent elevations in the G₂M peak, all MTA-treated patients showed characteristic changes in the early S region of the histogram in the post-perturbation period.

3.1.4. Acute myelogenous leukemia - collaborative study with Mount Sinai Medical Center

A study of the effects of AMSA and leukaphoresis in patients with acute myelogenous leukemia is continuing in collaboration with Dr. Michael Greenberg.

3.1.5. Kinetic perturbations in solid tumors - collaborative study with the Oncology Service at Walter Reed Medical Center

The effects of single doses of ara-C on DNA and RNA synthesis were studied in two patients to date. In one patient, no inhibition of DNA synthesis was observed following 200 mg/m² i.v. In a patient receiving 500 mg/m² i.v., a transient reduction in ³H-TdR incorporation was observed 2 hours after drug administration. This study is continuing. Once a clinical dose response curve has been established, interval optimization for ara-C plus adriamycin will be studied.

4. Publications

Cohen, M.H., Chretien, P.B., Ihde, D.C., Fossieck, B.E. Jr., Makuch, R., Bunn, P.A. Jr., Johnston, A.V., Shackney, S.E., Matthews, M.J., Lipson, S.D., Kenady, D.E., and Minna, J.D.: Thymosin fraction V and intensive combination chemotherapy. JAMA 241: 1813, 1979.

Shackney, S.E. and Skramstad, K.S.: A dynamic interpretation of multiparameter studies in the lymphomas. Amer. J. Clin. Path. 72: 756-764, 1979.

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Alabaster, O., Glaubiger, D.L., Shackney, S.E., Skramstad, K.S., Hamilton, V.T., Chen, R.F., and Bentley, S.A.: Electrolytic fluorochrome bleaching can affect the measurement of DNA content by flow cytometry. *J. Histochem. Cytochem.*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06512-04 CP
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Molecular Toxicology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Michael R. Boyd, M.D., Ph.D., Section Head Charles N. Statham, Ph.D., Cancer Expert John S. Dutcher, Ph.D., IPA Research Associate MTS, CPB, COP, DCT, NCI MTS, CPB, COP, DCT, NCI MTS, CPB, COP, DCT, NCI		
COOPERATING UNITS (if any) VA-NCI Medical Oncology Branch, COP, DCT, NCI (Dr. D. Ihde) Medicine Branch, COP, DCT, NCI (Dr. R. Young)		
LAB/BRANCH Clinical Pharmacology		
SECTION Molecular Toxicology		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Our efforts this year have been devoted primarily to the continuation and expansion of several projects described in detail in our FY 1979 Annual Report. We have applied our recently developed GC-MS assay for pentamethylmelamine to studies of the phase I pharmacology of this drug in cancer patients. Several lines of investigation are ongoing in the area of drug-induced pulmonary injury. Long-term toxicity and/or carcinogenicity studies centered primarily upon the lung-toxic furans are in progress. The roles of glutathione in drug-induced tissue injury continues to be a major interest. The role of the drug-metabolizing enzymes in the pathogenesis of chemical-induced injury to extrahepatic target cells, such as pulmonary Clara cells and renal proximal tubular cells, continues to be a major area of endeavor.		

I. Objectives of Research, Methods Employed:

A. Objectives

The primary goals of the Molecular Toxicology Section are: 1) to elucidate fundamental chemico-biologic interactions underlying target organ and target cell cytotoxicities and carcinogenicities by chemical agents, and 2) to provide consultation and expertise in the development and application of analytical methodologies required by clinical research programs of the Clinical Oncology Program, DCT, HCl.

B. Methods

A wide variety of instrumentation and techniques are employed in these investigations. Metabolic and dispositional studies utilize traditional methodologies and instrumental techniques including GC-mass spectrometry and high-pressure liquid chromatography. Toxicologic studies employ biochemical measures of cellular alterations, as well as morphologic measures such as high-resolution light microscopy, electron microscopy, and autoradiography.

II. Research Program Description: Specific Projects and Subprojects, Background, Accomplishments, Ongoing Activities, Future Plans:

A. Analytical and Clinical Pharmacology

Our efforts in this area have centered primarily upon the application of our recently developed GC-MS assay for pentamethylmelamine (PMM) to the investigation of the phase I clinical pharmacology of PMM in cancer patients.

PMM was given as a 1-hour i.v. infusion every 7 days until tumor progression. The drug was administered to 31 heavily pretreated patients with advanced solid tumors. Eight dose levels ranging from 30 to 1500 mg/m² were studied, with 3-7 patients treated at each level for a median of 3 (range 1-24) weekly infusions per patient. At each dose level, the pharmacokinetics of drug disappearance from plasma were studied in 1-4 patients. The data obtained were consistent with a two-compartment model of drug distribution, with a marginally dose-dependent terminal half-life of 43-195 minutes. Peak levels (observed at the end of infusion) were directly proportional to dose.

B. Molecular Toxicology

1. Biochemical mechanisms in drug-induced pulmonary disease.

Experimental investigations in this area are continuing along several avenues, including: 1) in vitro studies of the metabolic

activation of 4-ipomeanol in reconstituted systems containing purified components, including pulmonary cytochrome(s) P-450, NADPH-cytochrome P-450 reductase, and cytochrome b₅ (these studies are being done in collaboration with Dr. J. Bend and Dr. K. Philpot, NIEHS), 2) studies of the metabolic bases for the striking species-, age-, and sex-related differences in the *in vivo* target organ alkylation and cytotoxicity by 4-ipomeanol in laboratory animals, 3) studies of the metabolic and pharmacokinetic bases for alterations, by metabolic inducers and inhibitors, in the target organ specificity of metabolically activated alkylating agents, 4) studies of the metabolic activation (and cellular specificity thereof) of 4-ipomeanol in systems containing intact pulmonary cells (these systems include lung slices, isolated whole lungs, and partially resolved lung cell fractions, some of these studies are being pursued collaboratively with Dr. J. Bend, NIEHS), and 5) continuing metabolic, histopathologic, and autoradiographic studies with lung-toxic furans, including 3-methylfuran and perilla ketone (both of which occur naturally in the environment). Studies with other lung-toxic chemicals, including carbon tetrachloride and nitrofurantoin, have been described previously, and now are essentially completed. In continuing investigations, we are attempting to determine if any of our mechanistic models for chemical-induced lung injury can account for the pulmonary toxicities of clinically important anticancer drugs such as BCNU, procarbazine, and cyclophosphamide.

2. Physiology, pharmacology, and toxicology of the pulmonary Clara cell.

In ongoing investigations, we are continuing to explore the vulnerability of the Clara cell to cytotoxic and/or carcinogenic chemicals that require oxidative metabolic activation. These studies involve detailed histopathologic, autoradiographic, and metabolic investigations with a variety of pulmonary cytotoxins and carcinogens. We also are utilizing the selective destruction of pulmonary Clara cells with agents such as 4-ipomeanol as an approach to the study of the biochemical and physiologic role of the Clara cell in normal lung function.

3. Biochemical mechanisms in drug-induced renal disease.

In newly initiated studies, we are investigating the possible role of metabolism, and the possible protective role of GSH or other sulfhydryl compounds, in renal injury by methyl-CCNU, and are considering the possible modulatory roles of age- and sex-related differences in their metabolism, and how this may affect susceptibility to their renal toxicity.

4. Long-term toxicity and/or carcinogenicity studies.

Because of the possible importance of 4-ipomeanol, 3-methylfuran, and several other related furans as naturally occurring

toxicants, carcinogenicity studies of these compounds have been undertaken. (These studies are being pursued in collaboration with Drs. James and Elizabeth Miller, McArdle Laboratory for Cancer Research, Madison, Wisconsin.)

In addition, long-term inhalation toxicology studies are underway with 3-methylfuran (studies in collaboration with Dr. H. Witschi, Oak Ridge National Laboratory, Oak Ridge, Tennessee).

- b. Glutathione (GSH): Cellular homeostasis, role as protective factor in cytotoxicity by alkylating agents and reactive metabolites, formation of conjugates with reactive metabolites, and role of GSH transferases.

We have developed an HPLC method for the isolation of glutathione adducts formed with direct alkylating agents or with reactive drug metabolites. Structural studies, presently underway on such conjugates, should help elucidate the chemical identity of the alkylating metabolite(s) responsible for the pulmonary alkylation and toxicity by 4-ipomeanol *in vivo*. The possible role of pulmonary and hepatic GSH-transferase enzymes in promoting the detoxification of 4-ipomeanol also is being investigated. The possible general applicability of the GSH conjugate assay method to the analysis of adducts formed from a wide variety of alkylating agents and metabolites is being examined.

III. Significance to Biomedical Research and the Program of the Institute:

The potential utility of existing cancer chemotherapeutic agents is frequently hindered by dose-limiting toxicities to target organs such as the gastrointestinal tract, bone marrow, liver, kidney, and lung. Moreover, many antitumor agents in current use are also, in themselves, potent carcinogens. Thus, as the longer-term survival of patients on antitumor drug therapy becomes a reality, the problems of drug-induced secondary cancer paradoxically will assume greater significance.

The elucidation of molecular mechanisms whereby cytotoxic and/or carcinogenic chemicals exert their biologic effects hopefully will contribute to the improved usage of existing chemotherapeutic agents, and will help provide a more rational basis for the design of new drugs with optimal tumoricidal activity but minimal toxicity to normal tissues.

IV. Publications and Other Activities:

A. Abstracts

Haschek, W.M., Witschi, H.P., and Boyd, M.R.: Pulmonary toxicity of 3-methylfuran. Presented at the Annual Meeting, Amer. College of Veterinary Pathology, Denver, Colo., November 1979.

Haschek, W.M., Witschi, H.P., and Boyd, M.R.: Pulmonary and extra-pulmonary toxicity of 3-methylfuran in mice. Presented at the Annual Meeting, Soc. of Toxicology, Washington, D.C., March 1980. Toxicol. Appl. Pharmacol., in press.

Longo, N.S. and Boyd, M.R.: Sex differences in renal alkylation and toxicity by 4-ipomeanol in the mouse. Presented at the Annual Meeting, Soc. of Toxicology, Washington, D.C., March 1980. Toxicol. Appl. Pharmacol., in press.

Buckpitt, A.R. and Boyd, M.R.: Studies on the formation of GSH conjugates with reactive metabolites of 4-ipomeanol. Presented at the Annual meeting, Soc. of Toxicology, Washington, D.C., March 1980. Toxicol. Appl. Pharmacol., in press.

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B. Articles

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Boyd, M.R.: Biochemical mechanisms in pulmonary toxicities by furan derivatives. In *Reviews in Biochemical Toxicology*, E. Hodyson, J. Berd, and R. Philpot (Eds.), Elsevier/North Holland, New York, 1980, pp. 71-102.

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Dutcher, J.S., Jones, R.B., and Boyd, M.R.: A sensitive and specific assay for pentamethylmelamine in plasma: Application to preliminary pharmacokinetic studies. *Cancer Treat. Rep.*, 1980, in press.

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C. Invited Presentations at National and International Symposia

Ciba Foundation Symposium on "The Toxicological Significance of Interaction of Environmental Chemicals with Drug-Metabolizing Enzymes", October 23-25, 1979, London, England.

The Ninth Annual Meeting of the New England Pharmacology Society, January 25-26, 1980, Storrs, Connecticut.

The Second International Symposium on Biological Reactive Intermediates, July 14-17, 1980, Guildford, United Kingdom.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06513-04 CP		
PERIOD COVERED October 1, 1979, to September 30, 1980				
TITLE OF PROJECT (80 characters or less) Molecular Pharmacology of Antitumor Agents				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT				
<table style="width: 100%; border: none;"> <tr> <td style="width: 60%;"> PI: Bruce A. Chabner, M.D., Chief Ross Donehower, M.D., Clinical Associate Peter Ellims, M.D., Ph.D., Visiting Fellow Charles Erlichman, M.D., Visiting Fellow Chi-Ming Liang, Ph.D., Visiting Fellow Richard Schilsky, M.D., Clinical Associate Marc Lippman, M.D., Senior Investigator David Roselle, COSTEP University of Maryland School of Medicine James Drake, Biologist Brenda Bailey, Biologist Robert Do, Student Scientist Lawrence Tamarkin, Ph.D. </td> <td style="width: 40%; vertical-align: top; border: none;"> CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI MB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI University of Maryland NICHHD, NIH </td> </tr> </table>			PI: Bruce A. Chabner, M.D., Chief Ross Donehower, M.D., Clinical Associate Peter Ellims, M.D., Ph.D., Visiting Fellow Charles Erlichman, M.D., Visiting Fellow Chi-Ming Liang, Ph.D., Visiting Fellow Richard Schilsky, M.D., Clinical Associate Marc Lippman, M.D., Senior Investigator David Roselle, COSTEP University of Maryland School of Medicine James Drake, Biologist Brenda Bailey, Biologist Robert Do, Student Scientist Lawrence Tamarkin, Ph.D.	CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI MB, DCT, NCI CPB, DCT, NCI CPB, DCT, NCI University of Maryland NICHHD, NIH
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COOPERATING UNITS (if any) Pediatric Oncology Branch, Surgery Branch, Lab. of Chemical Pharmacology, Medicine Branch, Veterans Administration Oncology Branch, Lab. of Medicinal Chemistry and Biology				
LAB/BRANCH Clinical Pharmacology Branch				
SECTION Office of the Chief				
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland				
TOTAL MANYEARS: 6.5	PROFESSIONAL: 4	OTHER: 2.5		
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords) We have demonstrated for the first time the quantitative formation of methotrexate polyglutamates in human tumor cells. The key enzyme, dCMP deam- inase, which controls de novo synthesis of dCTP, has been purified to near homogeneity by a novel affinity procedure, and its regulation by nucleotide inhibitors and activators has been thoroughly characterized. Significant en- hancement of fluorouracil activation to FdUMP and enhanced incorporation into RNA have been demonstrated in the presence of the new pyrimidine antagonist PALA. A clinical protocol for treatment of malignant melanoma based on these findings has indicated a response rate (objective regressions, partial re- sponse, and complete response) of 3 of 9 patients evaluable thus far. Detailed pharmacokinetic studies of PALA have been completed using a novel competitive binding assay procedure. We have confirmed earlier studies which showed the pineal secretion, melatonin, suppresses development of breast tumors in rats exposed to the carcinogen DMBA. This suppression is correlated with suppres- sion of prolactin secretion by the pituitary and suggests that melatonin may exert an important influence over the prolactin secretory pattern in mammals.				

1. Our studies have conclusively demonstrated the formation of polyglutamates of methotrexate in human breast cancer cells in culture. MTX + G₁ and MTX + G₂ were first detected in these cells after six hours of incubation, became the predominant intracellular forms of MTX at 24 hours, and replaced the parent compound as the inhibitor of dihydrofolate reductase at this time point. We are continuing these studies with the aim of determining the relative retention and transport properties of the polyglutamates in breast cells, and their affinity of binding to dihydrofolate reductase. We are also studying their formation in resistant cell lines in collaboration with Dr. Ken Cowan of the Medicine Branch. (Schilsky, Bailey)
2. Studies of 5-fluorouracil (FU) interaction with the new pyrimidine antagonist PALA continue. We have shown a time- and dose-dependent synergism of cytotoxicity against sarcoma 180 cells in culture, with maximum effects at a PALA concentration of 300 μ M and with 12 hours of PALA preincubation. FU incorporation into RNA is dramatically enhanced, as is occupancy of FdUMP binding sites on thymidylate synthetase. We are determining effects on the free FdUMP pool as well. The PALA-FU combination has undergone phase I and phase II trials in the past year. Optimal doses of FU were 200 mg/m² per day on days 2, 3, 4, and 5, with PALA doses of 1000 mg/m² on days 1-5. No responses were observed in patients with colorectal carcinoma, but one partial response and two objective regressions have been observed in preliminary assessment of results in patients with malignant melanoma. (Erllichman, Donehower, Drake, Liang)
3. The pivotal enzyme, dCMP deaminase, which regulates the utilization of dUMP for TTP and dCTP synthesis, has been purified to near homogeneity by affinity chromatography on Blue Sepharose. Its molecular weight, pH dependence, substrate specificity, and regulation by triphosphates have been determined, and its activity with pharmacologic substrates also examined. Final purification and kinetic characterization will be completed this summer. (Ellims)
4. Characterization of the binding of PALA to aspartate transcarbamylase has been initiated. The enzyme has been disaggregated into subunits and a linear Scatchard binding plot has been observed in distinction to the hyperbolic curve seen with the aggregated enzyme. The kinetics of subunit binding and cooperativity are being investigated. (Erllichman)
5. The interaction of ara-C and 3-deazauridine has been studied. Synergistic cell kill against sarcoma 180 cells is observed with 4-6 hours preincubation with 3-deazaUR, with enhanced formation of ara-CTP. Accumulation of ara-CMP is observed with this combination; the reason for this finding is unclear. (Donehower, Drake, Chabner)
6. A repeat study has confirmed our earlier findings that melatonin suppresses the development of DMBA-induced breast cancer in the Sprague-Dawley rat. Protection was observed also in pinealectomized animals, although to a lesser extent, suggesting that the presence of an intact pineal is

important to this effect. The protective effect is associated with a fall in serum prolactin levels. Pinealectomy enhances the rate of tumor induction at low doses of DMBA, but this effect is not observed at high doses of DMBA since an enhancement is obscured by the high basal rate of tumor induction.

We have also found that melatonin administration leads to an acute drop in uterine estrogen and progesterone receptor activity in prepubertal, but not postpubertal, animals. This finding indicates that melatonin may exert its inhibitory effect on gonadal growth and maturation by producing end-organ insensitivity to estrogen or progesterone. (Chabner, Lippman, Do, Tamarkin, Roselle)

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murinson, D.S., Anderson, T., Schwartz, H.S., Myers, C.L., and Chabner, B.A.: Competitive binding radioassay for 5-fluorodeoxyuridine 5'-monophosphate in tissues. *Cancer Res.* 39: 2471-2475, 1979.

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PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Pharmacology and Clinical Application of Intraperitoneal Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles E. Myers, M.D., Head, Biochemical Pharmacology Section,
CPB, DCT, NCI
James L. Speyer, M.D., Clinical Associate
CPB, DCT, NCI
R. L. Dedrick, Ph.D., Biomed. Eng. & Instrum. Branch
DRS, NIH
J. M. Collins, Ph.D., Biomed. Eng. & Instrum. Branch
DRS, NIH
M. F. Brennan, M.D., Surgery Branch
DCT, NCI
P. H. Sugarbaker, M.D., Surgery Branch
DCT, NCI
Robert Ozols, M.D., Senior Investigator,
Medicine Branch
DCT, NCI
N. R. Dunnick, M.D., Senior Investigator, Diagnostic
Radiology Department
CC, NIH

COOPERATING UNITS (if any)

Medicine Branch, DCT, NCI, Biomedical Engineering & Instrumentation Branch,
DRS, NIH, Surgery Branch, DCT, NCI, Diagnostic Radiology Department, CC, NIH

LAB/BRANCH

Clinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NCI, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.5

PROFESSIONAL:

3

OTHER:

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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This laboratory has been involved in trying to improve the therapeutic efficacy of antineoplastic agents by administering them intraperitoneally via semi-permanent dialysis catheters to patients with tumor confined to the intra-abdominal space.

We have finished clinical trials of methotrexate and 5-FU and are currently conducting phase I trials of adriamycin and phase II trials of i.p. 5-FU. Pharmacologic studies are being conducted to define the transport of these drugs across the human peritoneum, measure the possible first-pass hepatic clearance and levels of hepatic perfusion, and correlate drug levels to clinical efficacy and toxicity.

1. 1.P. Methotrexate

With the assistance of Drs. Brennan and Balow, we have administered increasing doses of i.p. methotrexate with peripheral leucovorin rescue to five patients. Limiting toxicity consisted of apparent chemical peritonitis. There were no tumor responses. A ratio between peritoneal fluid and plasma drug levels of 25 was achieved.

2. 1.P. 5-FU

With the assistance of Drs. Sugarbaker, Brennan, Collins, and Dedrick, we have conducted phase I trials of 5-FU i.p. in ten patients. Dialysate concentrations ranged from 5 μ M to 5 mM and was administered for eight consecutive 4-hour exchanges every two weeks. Limiting toxicity was the same as systemically administered 5-FU. A very steep dose toxicity relationship was observed. There were two objective responses, both in prior treated ovarian patients. Peritoneal drug levels fell by a first order mechanism with a $t_{1/2}$ of 1.6 hours. At 4 hours, a ratio between peritoneal fluid and plasma drug levels of 298 was achieved.

Currently, the phase II trial is being conducted. Special concurrent investigations include 1) correlation of drug response to in vitro cell culture sensitivity assay in collaboration with Dr. Ozols, 2) correlation of drug levels to indocyanine green clearance, and 3) correlation of drug levels and toxicity with bone marrow EdUMP levels.

3. 1.P. Adriamycin

Preclinical studies have been conducted by Drs. Ozols, Myers, and Young, and Ms. Karen Wrotzinger to establish a model and rationale for i.p. adriamycin, a murine ovarian tumor model. Drs. Ozols and Myers with the assistance of Drs. Sugarbaker and Brennan are conducting phase I studies. Three patients have been treated with 5 mg to 60 mg per 2-liter dialysate. Fever and hyperamylasemia in several patients may indicate local toxicity. In two patients there was a significant decrease in ascites volume subsequent to drug administration. Preliminary measurements indicate the peritoneal fluid to plasma drug ratio achieved to be at least 200. Dr. Weltz worked on adapting an HPLC assay to analysis of these samples so that both parent compound and metabolites can be measured and correlations made separately to clinical efficacy and toxicity.

Publications

Murinson, D.C., Anderson, T., Schwartz, H.S., Myers, C.E., and Chabner, B.A.: Competitive binding radioassay for 5-fluorodeoxyuridine-5' monophosphate in tissues. *Cancer Res.* 39: 2471-2475, 1979.

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Speyer, J.L., Collins, J.M., Bedrick, R.L., Brennan, M.R., Londer, H., DeVita, V.T., and Myers, C.E.: Phase I and pharmacologic studies of intraperitoneal 5-FU. *Cancer Res.* 40: 567-572, 1980.

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Collins, J.M., Bedrick, R.L., King, F.G., Speyer, J.L., and Myers, C.E.: Non-linear pharmacokinetic models for 5-fluorouracil in man. *Clin. Pharm. Therap.*, in press.

Speyer, J.L. and Myers, C.E.: Intraperitoneal chemotherapy of ovarian cancer. In *Recent Advances in Clinical Oncology*. Whitehouse and Williams (Eds.), Churchill-Livingstone, Edinburgh, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06515-01 CP
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Biologic Response Modification: Free Radical Defenses and Their Modification by Nutrition		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Charles E. Myers, M.D., Head, Biochemical Pharmacology Section, CPB, DCT, NCI A. D. Katki, Ph.D., IPA Associate (Tufts University) CPB, DCT, NCI Johan G. S. Breed, M.D., Ph.D., Visiting Fellow CPB, DCT, NCI Raymond Greene, M.S., Pharmacist CPB, DCT, NCI Raymond Klecker, STRIDE Technician CPB, DCT, NCI Elizabeth Travis, Ph.D. ROB, DCT, NCI James Schwade, Ph.D. ROB, DCT, NCI Stephen Rosenberg, M.D., Ph.D., Chief, Surgery Branch, DCT, NCI Victor Ferrans, M.D., Chief, Ultrastructure, NHLBI Robert Bonow, M.D. NHLBI David Schwartz, M.D. NHLBI		
COOPERATING UNITS (if any) Radiation Oncology Branch and Surgery Branch, DCT, NCI, Ultrastructure, National Heart, Lung and Blood Institute.		
LAB/BRANCH Clinical Pharmacology		
SECTION Biochemical Pharmacology		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.9	PROFESSIONAL: 3.9	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINDRS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Our work with adriamycin cardiac toxicity has led us to re-examine the role of endogenous free radical defenses in moderating free radical damage. Since free radical damage is a process which plays a role in such diverse phenomena as aging, ionizing radiation damage, oxygen toxicity, carcinogenesis, and drug toxicity, we have tried to structure our investigation in such a way so as to yield results pertinent to this broad range of problems rather than to focus narrowly on anthracycline cardiac toxicity.		

I. Free Radical Defense Systems

The existing defenses against free radical attack have not been adequately described. We have, therefore, begun to examine these defenses in greater detail. In the process, we have found a relationship between selenium-dependent glutathione peroxidase and vitamin E which suggests that they interact positively. In addition, Dr. Katki discovered a new peroxidase bound to mitochondrial membrane and nuclear envelope which may play an important role in maintaining mitochondrial function and in protecting DNA from free radical damage. This new enzyme is of interest because it is membrane-bound, is not affected by selenium, and will utilize both hydrogen peroxide and lipid peroxides. We plan to examine the physiologic role of this enzyme further and to extend our examination of free radical defenses to other enzyme functions.

II. Role of Membrane Tocopherol

Vitamin E is a known in vitro free radical scavenger. In vivo it is found almost exclusively in cell membranes. In that setting it is not clear whether it operates as a radical scavenger or via other membrane-stabilizing mechanisms. In order to examine this process more thoroughly, Dr. Breed has developed an HPLC assay for tocopherol and one of its oxidation, tocopherol quinone.

These assays exceed by far the sensitivity of any previous technique, and we have been able to establish that baseline tocopherol quinone levels in cardiac tissue are 500-600 femtomoles per gram wet weight of tissue. This level increases to 1,000 femtomoles a few hours after DEM, suggesting that thiols are critical for the maintenance of vitamin E in the membrane.

What is most confusing is that supralethal radiation or adriamycin do not alter this pool. These results suggest minimal direct involvement of tocopherol in acute free radical attack. We intend to pursue this question further as it is of major theoretical importance.

III. Role of Glutathione in Radiation Damage

Although reduced glutathione is known to be a major defense against free radical attack, its role in radiation effects has really not been examined carefully. In collaboration with the radiation biologists under Dr. Travis, we hope to examine the role of glutathione in some detail. To that end, we have established assays for reduced glutathione, total thiols, and glutathione peroxidase, the major enzyme which utilizes glutathione to protect against free radical attack.

In addition, we have established mouse models where we can selectively delete each of the free radical defense enzymes either genetically or via diet. This includes superoxide dismutase, catalase, and glutathione peroxidase. These animals will allow us to test the role of glutathione in the presence and absence of each or all of the radical defenses.

Publications

Doroshow, J., Locker, G., and Myers, C.: Experimental animal models of adriamycin cardiotoxicity. *Cancer Treat. Rep.* 63: 855-860, 1979.

Locker, G.Y., Doroshow, J.H., Baldinger, J., Myers, C.E.: The relationship between dietary tocopherol and glutathione peroxidase activity in murine cardiac tissue. *Nutr. Rep. Internatl.* 19: 671-678, 1979

Doroshow, J.H., Locker, G.Y., and Myers, C.E.: The enzymatic defenses of the mouse heart against reactive oxygen metabolites. *J. Clin. Invest.* 65: 128-135, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03403-15 M
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Clinical Trials and Miscellaneous Clinical Investigations		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Robert C. Young Other: Bruce Chabner Charles Myers Richard Fisher Marc Lippman Dan Longo Robert Ozols Jacqueline Whang-Peng Vincent DeVita Richard Simon Steven Rosenberg Eli Glatstein Costan Berard	Chief Chief Sr Investigator Sr Investigator Sr Investigator Sr Investigator Sr Investigator Sr Investigator Director Chief Chief Chief Chief Sr Investigator	M NCI CP NCI M NCI M NCI M NCI M NCI M NCI M NCI NCI BR NCI S NCI RO NCI LP NCI
COOPERATING UNITS (if any) Radiation Oncology Branch, NCI; Clinical Pharmacology Branch, NCI; Biometrics Research Branch, NCI; Surgery Branch, NCI; Immunology Branch, NCI; Laboratory of Clinical Investigation, NIAID		
LAB/BRANCH Medicine Branch		
SECTION		
INSTITUTE AND LOCATION Clinical Oncology Program, NCI, Bethesda, Maryland 20205		
TOTAL MANYEARS: 26	PROFESSIONAL: 19.5	OTHER: 7.5
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The Medicine Branch is a major clinical facility of the National Cancer Institute. Its activities are divided between <u>clinical therapeutic trials</u> in patients with cancer and <u>clinically related laboratory research</u> . Clinical trials of cancer treatment in the following diseases are currently underway: breast cancer, ovarian cancer, Hodgkin's disease, non-Hodgkin's lymphomas, testicular tumors, melanoma, cervical carcinoma, and endometrial carcinoma. In addition, <u>Phase I-II</u> <u>clinical trials</u> have been completed this year on the following new experimental agents or combinations: AMSA, PALA and 5-FU, chlorozotocin, ICRF-187, and penta- methylmelamine. Phase I-II trials continue on AZQ, 13-cis-retinoic acid, intra- peritoneal chemotherapy of 5-FU and adriamycin. Additional summaries of clinical studies are summarized under reports entitled " <u>Clinical Program in Breast Carcino-</u> <u>ma.</u> " Laboratory research of the branch is summarized under reports entitled " <u>Clinical Pharmacology of Antineoplastic Agents, Kinetics of Cellular Proliferation,</u>		

Other (contd):	Susan Hubbard	Chemo Res Nurse	M	NCI
	Dianne Howser	Chemo Res Nurse	M	NCI
	Audrey Barlock	Chemo Res Nurse	M	NCI
	Jane Cassidy	Chemo Res Nurse	M	NCI
	First and Second Year Clin Associates		M	NCI

Summary of Work (Contd):

Cytogenetic Studies, Immunologic Aspects of Cancer, Mechanisms of Hormone Dependence of Human Malignancy, and Genetic Regulation of the Immune Response."

Major Accomplishments in 1979-1980:

Ovarian Carcinoma: Established:

- 1) The activity of the Hexa-CAF combination in advanced ovarian cancer has been confirmed by Dutch investigators and is now being compared in a prospective trial with CAP (cyclophosphamide, adriamycin, platinum) at the Princess Margaret Hospital, Toronto, Canada. The median survival of 29 months remains the longest yet reported for advanced epithelial ovarian cancer.
- 2) The CHex-UP combination (cyclophosphamide, hexamethylmelamine, and 5-fluorouracil and cis-platinum) currently under study appears to have a complete remission rate even higher than Hexa-CAF (52% vs 33%) with approximately 50 patients now under study.
- 3) Over 100 patients with "early" ovarian carcinoma have now had staging laparotomy prior to therapy at Ovarian Cancer Study Group institutions. 21/67 (32%) of patients referred apparently free of disease had residual disease identified by careful staging. Manuscript on these findings is now in preparation.
- 4) Extensive experience with the human ovarian cancer stem cell assay has been completed. Over 60 patients have been studied. Clinical correlation with assay findings exceeds 90% for inactive agents and exceeds 64% for those deemed active in the assay.
- 5) Intraperitoneal chemotherapy coupled with simultaneous stem cell assay of various doses of chemotherapy has allowed us to define doses necessary for effective therapy. Three types of human ovarian cancer cells are now recognized. The first is sensitive to the drug at doses of chemotherapy which can be achieved by conventional administration. The second are cells resistant at conventional doses but sensitive at the higher doses which can be reached by intraperitoneal administration. The third group are resistant to the drug at all concentrations. This data will have important implications in our selection of future chemotherapy in ovarian cancer.

Ovarian Carcinoma: Published:

Ovarian cancer patients have a unique B cell defect; cis-platinum is an effective drug in advanced ovarian cancer; histologic grade has prognostic importance in response to chemotherapy in advanced disease; reviews of the status of staging and treatment of ovarian cancer; the potential etiologic role of cosmetic talc in ovarian cancer; strategies for effective management for early ovarian cancer; comprehensive review of chemotherapy of gynecologic malignancies.

Hodgkin's Disease: Established:

- 1) The 10 year followup of 198 patients with advanced Hodgkin's disease was published this year. Complete remission rate is 80%. 68% of the complete remitters remain continuously disease free at 10 years. Overall survival for those achieving remission is 82% at 5 years and 73% at 10 years. These results show that advanced Hodgkin's disease is curable with combination chemotherapy.
- 2) A persistent defect in T cell function occurs in Hodgkin's disease patients who have been cured of their disease. This defect appears intrinsic to the disease and is not seen in patients with diffuse histiocytic lymphoma treated with similar chemotherapy. The defect appears to be related to abnormal responses to normal suppression T cells in patients with Hodgkin's disease.
- 3) That prolonged disease free survival can be achieved in Hodgkin's disease following MOPP reinduction after first relapse. Median survival of all patients so treated exceeds 5 years from the time of first relapse.

Hodgkin's Disease: Published:

Curability of advanced Hodgkin's disease; persistent immunologic abnormalities in long term survivors; comprehensive review of the chemotherapy of Hodgkin's disease.

Non-Hodgkin's Lymphoma: Established:

- 1) Comprehensive review of 473 patients with non-Hodgkin's lymphoma treated at the NCI over a 22 year period has been completed this year and has resulted thus far in 7 publications. Highlights of these comprehensive studies include: the recognition of the frequency of histologic conversion in the "good prognosis" lymphomas; the aggressive nature of nodular histiocytic lymphoma; comparisons of alternative histologic classifications; the staging of non-Hodgkin's lymphomas; and prognostic factors in non-Hodgkin's lymphoma.

- 2) The characterization of the natural history of divergent histologies which present at initial diagnosis.
- 3) The importance of histologic conversion on the relapse frequency, response to therapy, and subsequent survival of patients with non-Hodgkin's lymphoma. Nodular lymphomas which convert to diffuse histologies have a worse prognosis and shorter survival unless induced into a complete remission by aggressive chemotherapy.
- 4) Description of the central nervous system complications of non-Hodgkin's lymphoma and characterization of the population at high risk for CNS lymphoma. Successfully defined the population requiring prophylactic therapy.
- 5) Gallium Scanning in non-Hodgkin's lymphoma: comprehensive review of our 10 year experience staging the lymphomas shows that routine use of the gallium scan in staging is not cost effective and uncommonly is positive when other studies are not.

Non-Hodgkin's Lymphoma: Published:

Changing concepts of the lymphomas; CNS lymphoma, a complication of diffuse histologies; a prospective trial comparing total body irradiation plus combination chemotherapy with combination chemotherapy alone in the management of "good prognosis" lymphomas; activity of VP-16 in the treatment of resistant non-Hodgkin's lymphomas.

Breast Carcinoma: Established:

- 1) A prospective randomized trial of tamoxifen + halotestin is nearing completion (112 patients) and suggests that the combination hormonal approach is superior in response rate to tamoxifen alone. Analysis by receptor status is underway.
- 2) Preliminary analysis of a prospective randomized trial of chemotherapy + hormonal synchronization suggests that ER+ patients may benefit from the synchronization.
- 3) The important antitumor properties of the biological response modifier - cis-retinoic acid - are being tested in a Phase II trial in advanced breast cancer.
- 4) More detailed discussion of the clinical breast programs in breast cancer are discussed under the section entitled "Clinical Programs in Breast Cancer."

Melanoma: Established:

- 1) Prospective trial randomizing poor prognosis Stage I and II melanoma patients to MeCCNU, BCG, and BCG + tumor cell vaccine has been closed to new accrual. Preliminary analysis indicates there is no difference in relapse free survival among the three groups.
- 2) A comprehensive review of the presence of steroid receptors in malignant melanoma and their potential role in response to hormonal therapy was completed this year as was a review of the endocrine aspects of malignant melanoma.
- 3) Other melanoma studies are discussed under the section entitled "Kinetics of Cellular Proliferation."

Published:

Phase II study of high dose methotrexate in patients with advanced malignant melanoma.

Sarcomas: Established:

- 1) The apparent lack of improvement in survival in osteogenic sarcoma from high dose methotrexate but the improved survival related to repeated surgical resection.
- 2) A comprehensive review of the clinical features of hepatic angiosarcoma.
- 3) Description of an anaplastic sarcoma arising in a benign chondroblastoma.

Genitourinary Neoplasms: Established:

- 1) The apparent benefit of cytoreductive surgery prior to aggressive chemotherapy in the treatment of non-seminomatous testicular cancer.
- 2) Correlation of computerized tomography and serum tumor markers in metastatic retroperitoneal testicular cancer.

Genitourinary Neoplasms: Published

Reviews of the role of chemotherapy in the management of bladder cancer and other genitourinary neoplasms. Hypomagnesemia and renal magnesium wasting in patients receiving cis-platinum.

Other major accomplishments in 1979-1980 will be found in the individual project title reports for the Medicine Branch.

Z01 CM 03420-11 M
Z01 CM 03404-09 M
Z01 CM 06700-07 M
Z01 CM 06702-05 M
Z01 CM 06119-11 M
Z01 CM 06708-01 M

Publications:

OVARIAN CANCER:

1. Mandell, G.L., Bostick, F., Young, R.C., and Fisher, R.I.: Ovarian cancer: A solid tumor with normal cellular immunity but abnormal B cell function. *Am. J. Med.* 66: 621-624, 1979.
2. Young, R.C., Von Hoff, D.D., Gormley, P., Makuch, R., Cassidy, J., Howser, D.M., and Bull, J.M.: Cis-Diamminedichloroplatinum II for the treatment of advanced ovarian cancer. *Cancer Treat. Rep.* 63: 1539-1544, 1979.
3. Von Hoff, D.D., Schilsky, R., Reichert, C., Reddick, R., Rozenzweig, M., Young, R.C., Muggia, F.M.: Non-renal toxicities of cis-diammine-dichloroplatinum II (NSC 119875). *Cancer Treat. Rep.* In press.
4. Young, R.C.: Ovarian cancer: Current status on controlled studies in staging and therapy. Veronesi, V., Perussia, A., Di Re, F. and Emanuelli, H. (Eds). Le Neoplasie Dell'Apparato Genitale Femminile. Milano, Italy, Casa Editrice Amrosiana, 1978, pp. 289-296.
5. Ozols, R.F., Garvin, A.J., Costan, J., Simon, R.M., and Young, R.C.: Histologic grade in advanced ovarian cancer. *Cancer Treat Rep.* 63: 255-263, 1979.
6. Young, R.C.: Ovarian carcinoma: An optimistic epilogue. *Cancer Treat. Rep.* 63: 333-337, 1979.
7. Longo, D.L. and Young, R.C.: Cosmetic talc and ovarian carcinoma. *Lancet* II: 349-351, 1979.
8. Young, R.C.: A strategy for effective management for early ovarian carcinoma. In Adjuvant Therapy of Cancer II, Grune and Stratton, 1979, pp. 467-474.

9. Ozols, R.F. and Young, R.C.: The management of advanced ovarian cancer. In Principles of Cancer Treatment, McGraw-Hill. In press.
10. Lewis, B.J. and Young, R.C.: Adjuvant and first line chemotherapy for ovarian cancer. International Symposium on Ovarian Cancer C.E. Newman (Ed), Pergamon Press. In press.
11. Lewis, B.J. and Young, R.C.: Ovarian cancer: future approaches to the development of treatment. International Symposium on Ovarian Cancer. C.E. Newman (Ed), Pergamon Press. In press.
12. Young, R.C.: Gynecologic malignancies. In Cancer Chemotherapy 1979. H.M. Pinedo (Ed). Amsterdam: Excerpta Medica, 1979, pp. 340-375.
13. Ozols, R.F., Willson, J.K.V., Young, R.C.: Human ovarian cancer colony formation: growth from malignant washings and pharmacologic applications. In Cloning of Human Tumor Stem Cells. S.E. Salmon (Ed). In press.
14. Dunnick, N.R., Fisher, R.I., Chu, E.W., and Young, R.C.: Percutaneous aspiration of retroperitoneal lymph nodes in patients with ovarian cancer. *Am. J. Radio.* In press.
15. Ozols, R.F., Garvin, A.J., Costa, J., Simon, R.M., Young, R.C.: Advanced ovarian cancer: correlation of histologic grade with response to therapy. *Cancer* 45: 572-581, 1980.
16. Young, R.C.: Chemotherapy of the gynecologic malignancies. In Cancer Chemotherapy 1980. H.M. Pinedo (Ed). Amsterdam: Excerpta Medica, 1980. In press.
17. Ozols, R.F., Willson, J.K.V., Grotzinger, K.R., Young, R.C.: Cloning of human ovarian cancer cells in soft agar from malignant effusions and peritoneal washings. *Cancer Research*. In press.

HODGKIN'S DISEASE:

18. DeVita, V.T., Simon, R.M., Hubbard, S.M., Young, R.C., Berard, C.W., Moxley, H.J., Frei, E., III, Carbone, P.P., Canellos, G.P.: On the curability of advanced Hodgkin's disease with chemotherapy: long term followup of MOPP treated patients at the NCI and the influence of disease variables on prognosis. *Ann. Int. Med.* In press.
19. Fisher, R.I., DeVita, V.T., Bostick, F., Vanhaelen, C., Howser, D.M., Hubbard, S.M., Young, R.C.: Persistent immunologic abnormalities in long-term survivors of advanced Hodgkin's disease. *Ann. Int. Med.* In press.

20. Young, R.C. and DeVita, V.T.: Chemotherapy of Hodgkin's disease. In Clinics in Haematology. W.B. Saunders, Co. 8: 625-644, 1979.
21. Fisher, R.I., DeVita, V.T., Hubbard, S.P., Simon, R.M., Young, R.C.: Prolonged disease-free survival in Hodgkin's disease following reinduction with MOPP after first relapse. *Ann. Int.Med.* 90: 761-763, 1979.
22. DeVita, V.T., Lewis, B.J., Rozencweig, M., and Muggia, F.M.: The chemotherapy of Hodgkin's disease: Past experiences and future direction. *Cancer*, In press.
23. Lewis, B.J. and DeVita, V.T.: Hodgkin's disease. Invited paper for *Southern Medical Journal*. In press.

NON-HODGKIN'S LYMPHOMA:

24. Young, R.C., Howser, D.M., Anderson, T., Jaffe, E., DeVita, V.T.: CNS lymphoma: a complication of diffuse histologies. CNS Complications of Malignant Disease. Kay and Whitehouse (Eds). MacMillan Press, pp. 121-131, 1980.
25. Young, R.C., Howser, D.M., Anderson, T., Fisher, R.I., Jaffe, E., DeVita, V.T.: Central nervous system complications of non-Hodgkin's lymphoma: The potential role for prophylactic therapy. *Am. J. Med.* 66: 435-443, 1979.
26. DeVita, V.T., Glatstein, E., Young, R.C., Ziegler, J., Wiernik, P.: Changing concepts: The lymphomas. In *Adjuvant Therapy of Cancer II*. Grune and Stratton, pp 173-190, 1979.
27. Longo, D.L., Schilsky, R.L., Blei, L., Cano, R., Johnston G.S., Young, R.C.: Gallium-67 scanning has limited usefulness in staging patients with non-Hodgkin's lymphoma. *Am. J. Med.* 68: 695-700, 1980.
28. Brereton, H.D., Young, R.C., Longo, D.L., Kirkland, L.R., Berard, C.W., Jaffe, E.S., DeVita, V.T., Johnson, R.E.: Comparison between combination chemotherapy and total body irradiation plus combination chemotherapy in non-Hodgkin's lymphoma. *Cancer* 43: 2227-2231, 1979.
29. Garvin, A.J., Simon, R., Young, R.C., DeVita, V.T., Berard, C.W.: The Rappaport classification of non-Hodgkin's lymphoma: a closer look using other proposed classifications. *Seminars in Onc.* In press.
30. Chabner, B.A., Fisher, R.I., Young, R.C., DeVita, V.T.: Staging of non-Hodgkin's lymphoma. *Sem. Onc.* In press.

31. Osborne, C.K, Norton, L., Young, R.C., Garvin, A.J., Simon, R.M., Berard C.W., DeVita, V.T.: Nodular histiocytic lymphoma: an aggressive nodular lymphoma with potential for prolonged disease free survival. Blood. In press.
32. Fisher, R.I., Jones, R.B., DeVita, V.T., Simon, R.M., Garvin, A.J., Berard, C.W., Young, R.C.: Natural history of malignant lymphomas with divergent histologies at staging evaluation. Cancer. In press.
33. Come, S.E., Jaffe, E.S., Anderson, J.C., Mann, R.B., Johnson, B.L., DeVita, V.T., Young, R.C.: Leukemic progression of non-Hodgkin's lymphoma: clinicopathologic features and therapeutic implications. Am. J. Med. In press.
34. Come, S.E. and Chabner, B.A.: Staging in non-Hodgkin's lymphoma: Approach, results and relationship to histopathology. Clinics Hematol. 8: 645-656, 1979.
35. Chabner, B.A.: Nodular non-Hodgkin's lymphoma. The case for watchful waiting. Ann. Intern. Med. 90: 115-117, 1979.
36. Lewis, B.J. and DeVita, V.T.: Combination therapy of the lymphomas. Invited paper for Seminars in Hematology, in press.
37. Lewis, B.J. and DeVita, V.T.: The lymphomas - recent progress. Drug Therapy. In press.
38. Popp, M.B., Fisher, R.I., Simon, R.M., and Brennan, M.F.: A prospective randomized study of adjuvant parenteral nutrition in the treatment of diffuse lymphoma. I. Effect on drug tolerance. Cancer Treat. Rep. In press.

BREAST CARCINOMA:

See Section entitled "Clinical Program in Breast Carcinoma"

MELANOMA:

39. Fisher, R.I. and Lippman, M.E.: Steroid receptors in malignant melanoma. In Thompson, B. and Lippman, M. (Eds.). Steroid Receptors and the Management of Cancer. Cleveland, CRC Press, Vol 1, 1979, pp 197-204.

40. Fisher, R.I., Lippman, M.E.: Endocrine aspects of malignant melanoma. In Rose, D.P. (Ed.). Endocrinology of Cancer. CRC Press, Cleveland. Vol. II: 87-94, 1979.
41. Fisher, R.I., Chabner, B.A., Myers, C.E.: Phase II study of high dose methotrexate in patients with advanced malignant melanoma. *Cancer Treat. Rep.* 63: 147-148, 1979.

SARCOMAS:

42. Rosenberg, S.A., Chabner, B.A., Young, R.C., Seipp, C., Levine, A.S., Costa, J., Hanson, T.A., Head, G., Simon, R.M.: The treatment of osteogenic sarcoma. I. The effect of adjuvant high-dose methotrexate following amputation. *Cancer Treat. Rep.* 63: 739-751, 1979.
43. Locker, G.Y., Doroshow, J.H., Zwelling, L.A., Chabner, B.A.: The clinical features of hepatic angiosarcoma: A report of four cases and a review of the English literature. *Medicine* 58: 48-64, 1979.
44. Gelman, E.P., Costa, J., Myers, C.E.: Anaplastic sarcoma arising in a benign chondroblastoma. *J. Bone Joint Surg.* In press.

GENITOURINARY NEOPLASMS:

45. Anderson, T., Waldmann, T.A., Javadpour, N., Glatstein, E.: Testicular germ cell neoplasms: recent advances in diagnosis and therapy. *Ann. Int. Med.* 90: 373-385, 1979.
46. Dunnick, N.R., Anderson, T.: Distant metastases to bone from carcinoma of the bladder. *Am. J. Roentgenol.* 132: 469-470, 1979.
47. Anderson, T., Makuch, R., Bunn, P., Ritch, P., Radice, P., Huberman, M., Young, R.C.: Peritoneoscopy: utilization in evaluation of hepatic metastases. *EORTC Monograph*. In press.
48. Anderson, T., Javadpour, N., Schilsky, R., Barlock, A., Young, R.C.: Chemotherapy of testicular cancer: current status of the National Cancer Institute combined modality trial. *Cancer Treat Rep.* In press.
49. Schilsky, R.L., Anderson, T.: Hypomagnesemia and renal magnesium wasting in patients receiving cis-platinum. *Ann. Int. Med.* In press.

MISCELLANEOUS:

50. Hande, K.R., Chretien, P.B., Elias, E.G., Goldberg, N.H., Brooks, A.E., Chabner, B.A.: Thirty-six-hour preoperative high-dose methotrexate infusion in patients with squamous carcinoma of the head and neck. *Med. Pediatric Oncol.* 6: 39-45, 1979.

51. Schilsky, R.L., Lewis, B.J., Sherins, R.J., and Young, R.C.: Gonadal dysfunction in patients receiving chemotherapy for cancer. *Ann. Int. Med.* In press.
52. Lewis, B.J. and DeVita, V.T.: Cancer chemotherapeutic agents in the last 10 years - combination chemotherapy programs. *Cancerologia Clinica*, in press.
53. Longo, D.L. and Witherspoon, J.M.: Focal neurologic symptoms in hypercalcemia. *Neurology* 32: 200-201, 1980.
54. Hubbard, S.M.: The foreign exchange nurse visitor program: an international program for cancer nurses. *Cancer Nursing* 5: 351-352, 1979.
55. Hubbard, S.M.: Neoplasia Chapter 5. In Jones, D.A., et al (Eds.). Medical Surgical Nursing: A Conceptual Approach. McGraw-Hill, New York, 1980, in press.
56. Hubbard, S.M.: Chapter 14: Chemotherapy and the cancer nurse. In Marino, L.B. (Ed). Cancer Nursing. Mosby Co., New York, in press.
57. Hubbard, S.M. and DeVita, V.T.: Breast cancer. In Guerier J.P. (Ed.). *Merit Students Encyclopedia*. New York, McMillan Educational Corp., in press.
58. Hubbard, S.M. and Donehower, M.G.: The nurse in a cancer research setting. *Seminars in Onc.* 7: 9-17, 1980.
59. Hubbard, S.M.: Effective communication through oral presentation. *Onc. Nursing Forum* 7: 37-40, 1980.
60. Gortner, S.R., Cross, S., Rugg, J., Hubbard, S.M., and McGuire, D.: The three faces of teaching research: In *Issues in Posing: Nurses Challenge for Change*. ANA Publication, 1979, pp 117-124.
61. Barlock, A.L., Howser, D.M., and Hubbard, S.: Nursing management of adriamycin extravasation. *Amer. J. Nurs.*, January, 1979.
62. Vermess, M., Doppman, J.L., Sugarbaker, P., Fisher, R.I., Chatterji, D.C., Luetzler, J., Grimes, G., Girton, M., and Adamson, R.H.: Clinical trials with a newly developed intravenous liposoluble contrast material for computerized tomographic examination of the liver and spleen. *Radiology*, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03404-09 M
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Immunologic Aspects of Cancer		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Richard Fisher Other: Christian Vanhaelen Zdenko Krizan Frieda Bostick Mark Greene Dean Mann Elaine Jaffe	Sr Investigator Visiting Fellow Visiting Fellow Technician Sr Investigator Sr Investigator Sr Investigator	M NCI M NCI M NCI M NCI E NCI I NCI LP NCI
COOPERATING UNITS (if any) Immunology Branch, DCBD, NCI; Laboratory of Pathology, DCBD, NCI		
LAB/BRANCH Medicine Branch		
SECTION		
INSTITUTE AND LOCATION Clinical Oncology Program, NCI, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Recent studies in this laboratory have suggested that patients with Hodgkin's disease may have significant abnormalities of immune function unassociated with the presence of active disease. Assays of non-specific immunity are abnormal in patients with Hodgkin's disease cured by MOPP chemotherapy. These abnormalities cannot be attributed to the chemotherapy since they are not present in patients with diffuse histiocytic lymphoma cured by similar chemotherapy regimens. T lymphocytes in both untreated and cured patients with Hodgkin's disease have increased sensitivity to suppression by normal immune regulatory cells. This has been shown for both a macrophage suppressor cell and a T cell suppressor induced by concanavalin A. Both suppressor systems operate independently of prostaglandin E. A new serologic specificity closely linked to the HLA-Dr locus has been observed in cured Hodgkin's patients. Studies are in progress to define the genetics of these immune abnormalities by studying families of affected patients.		

A murine model of ovarian cancer is being studied. The inability to generate cellular immunity against the tumor has been explained by the tumor's lack of H-2 antigens. Successful immunotherapy has been shown to be dependent on the agent utilized, dose, route, and schedule. Synergism between chemotherapy and immunotherapy is observed. These studies provide a basis for a Phase I human ovarian immunotherapy trial.

Publications:

1. Mandell, G.L., Fisher, R.I., Bostick, F., and Young, R.C.: Ovarian cancer: A solid tumor with normal cellular immunity but abnormal B cell function. *Amer. J. Med.* 66: 621-624, 1979.
2. Ozols, R.F., Grotzinger, K.R., Fisher, R.I., Myers, C.E., and Young, R.C.: Kinetic characterization and response to chemotherapy in a transplantable murine ovarian cancer. *Cancer Res.* 39: 3202-3208, 1979.
3. Ozols, R.F., Locker, G.Y., Doroshow, J.H., Grotzinger, K.R., Myers, C.E., Fisher, R.I., and Young, R.C.: Chemotherapy for murine ovarian cancer: A rationale for i.p. therapy with adriamycin. *Cancer Treat. Rep.* 63: 269-274, 1979.
4. Fisher, R.I., Mandell, G.L., Bostick, F., McMenamin, M.G., and Anderson, T.: Chlorozotocin, an antitumor agent lacking bone marrow toxicity at therapeutic doses: Effects on lymphocyte subpopulations in mice. *Clin. Exp. Immunol.* 39: 416-425, 1980.
5. Fisher, R.I., DeVita, V.T., Bostick, F., Vanhaelen, C., Howser, D.M., Hubbard, S.M., and Young, R.C.: Persistent immunologic abnormalities in long term survivors of advanced Hodgkin's disease. *Ann. Intern. Med.* 92: 595-599, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03420-11 M
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Kinetics of Cellular Proliferation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Robert C. Young Other: Robert Ozols Amy Newburger Bruce Chabner Charles Myers Stanley Shackney Richard Fisher Karen Grotzinger Wilma McKoy	Chief Sr Investigator Staff Scientist Chief Sr Investigator Sr Investigator Med Technologist Med Technologist	M NCI M NCI M NCI CP NCI M NCI CP NCI M NCI M NCI M NCI
COOPERATING UNITS (if any) Clinical Pharmacology Branch, NCI		
LAB/BRANCH Medicine Branch		
SECTION Cellular Kinetics Section		
INSTITUTE AND LOCATION Clinical Oncology Program, NCI, Bethesda, Maryland 20205		
TOTAL MANYEARS: 6.0	PROFESSIONAL: 3.0	OTHER: 2.50
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The primary goal of the <u>Cellular Kinetics</u> Section is to develop a better understanding of the kinetic behavior of normal and tumorous tissues and to use such information to design optimal forms of chemotherapy. Toward this end both animal studies and studies in patients have been undertaken. I. <u>Animal Studies</u> This year <u>alterations</u> in <u>DNA synthesis induced by chemotherapy</u> in normal and tumorous murine tissues have been completed and published. Cellular kinetic studies in an <u>ovarian carcinoma murine tumor model</u> have been completed and published. Pharmacokinetics of <u>intraperitoneal administration of methotrexate, 5-fluorouracil, and adriamycin</u> have been related to cellular kinetics, tissue penetration and systemic toxicity for the first time. Other animal studies		

include: the kinetic effects of tamoxifen followed by estrogen, tamoxifen or estrogen alone have been studied in the DMBA induced breast cancer in Sprague-Dawley rats. The tumors were studied by autoradiography, TdR incorporation, and flow cytometry. No evidence of recruitment or synchronization with estrogen, following tamoxifen occurred at 1,2 or 3 days post-therapy.

The kinetic perturbation in normal mouse bone marrow following hydroxyurea has been completed this year. The study shows that the marrow is repopulated not only by cells in the subendosteal region, but also by recruitment of more slowly cycling cells in the central region, normally considered the zone of granulocyte differentiation. Recovery of the normal kinetic gradient occurred by 96 hours. Manuscript has been submitted.

II. Kinetic Studies in Patients

Ovarian Cancer: After methotrexate and adriamycin therapy kinetic studies have been carried out using the human tumor stem cell assay, assays on the sensitivity of human ovarian cancer to alkylating agents, 5-FU, adriamycin and cis-platinum have been studied and related to attainable in vivo concentrations. Studies on the cytotoxic drug transport in human ovarian cancer have also been completed.

We have demonstrated that human ovarian cancer cells can be cloned in soft agar from 80% of cytologically malignant effusions and peritoneal washings. We have thus been able to determine the patterns of in vitro sensitivity of ovarian cancer cells to adriamycin and to chemotherapeutic agents used in the treatment of refractory ovarian cancer. These in vitro studies have provided the rationale for the administration of intraperitoneal adriamycin in selected ovarian cancer patients. We have established that amphotericin B is not effective in overcoming drug resistance in vitro.

We have also demonstrated that human endometrial cancer, melanoma and gastrointestinal tumors will clone in this assay. This assay allows us to study 1) the biology of human tumor growth; 2) to individualize chemotherapy on the basis of in vitro sensitivity to various antitumor agents; 3) to determine in vitro the effect of biologic modifiers on tumor growth and on the potentiation of chemotherapeutic agents; 4) to study the mechanisms of drug resistance and possible ways to overcome this resistance and 5) to screen for new antineoplastic agents in a tumor system which may be more predictive than traditional drug screening techniques.

Malignant Melanoma: Patients with Stage III malignant melanoma with cutaneous or subcutaneous metastases are randomized to receive methotrexate for 48 hours (5 mg/m² PO q6h) followed by leucovorin rescue, followed by MeCCNU (250 mg/m²) or the same dose of MeCCNU alone. This protocol is designed to examine the in vivo effects of MeCCNU and to test the feasibility of in vivo synchronization of malignant melanoma. Patients have serial biopsies of cutaneous lesions obtained for H³-TdR labeling indices and single cell suspensions are made for

flow cytometry. Labeling index changes failed to predict for clinical response but a specific alteration of the DNA histogram was useful. Persistence of altered tumor DNA histograms at one week after MeCCNU therapy correlates with clinical response in metastatic malignant melanoma. These alterations in DNA histograms may help to define the timing of drug action and aid in designing chemotherapy schedules in melanoma.

Studies with cultured murine melanomas (B16 and Cloudman CCL-53, IA and Mel) and human melanomas (FCC-1 and HM-1) to look for melatonin receptors and responsiveness to MSH as measured by increased tyrosinase production. During these studies an aberrant human melanoma protein analogous to B16 murine melanoma has been identified, isolated and partially purified and is now being used to generate an antibody as a potential tool for the early diagnosis of melanoma.

Breast Cancer: Patients with cutaneous metastases of breast cancer on MB-160 have also participated in a similar kinetic protocol. Biopsies of the cutaneous lesions are obtained pre-treatment and on days 5 and 7 for ^3H -TdR labeling indices, and PDP indices. No predictable pattern of changes occurred in the PDP or ^3H -TdR labeling indices.

Additional Studies: These include the pharmacokinetics and cellular kinetic effects of intraperitoneal methotrexate, 5-FU and adriamycin in patients with ovarian cancer with ascites.

Publications:

1. Ozols, R.F., Locker, G.Y., Doroshow, J.H., Grotzinger, K.R., Myers, C.E., Fisher, R.I., and Young, R.C.: Chemotherapy of murine ovarian cancer: a rationale for intraperitoneal therapy with adriamycin. *Cancer Treat Rep.* 63: 269-273, 1979.
2. Ozols, R.F., Grotzinger, K.R., Fisher, R.I., Myers, C.E., and Young, R.C.: Kinetic characterization and response to chemotherapy in a transplantable murine ovarian cancer. *Cancer Res.* 39: 3202-3208, 1979.
3. Ozols, R.F., Locker, G.Y., Doroshow, J.H., Grotzinger, K.R., Myers, C.E., and Young, R.C.: Adriamycin treatment of ovarian cancer: Pharmacokinetics, tissue penetration, and rationale for intraperitoneal administration. *Cancer Res.* 39: 3209-3214, 1979.
4. Newburger, A.E. and Weinstein, G.D.: Cell proliferation patterns in human malignant melanoma, in vivo. *Cancer*, in press.
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6. Hearing, V., Newburger, A.E., Ekel, T.M., and Montague, P.: Malignant melanoma- Abnormal proteins synthesized in murine and normal tissues. Clin. Res., May, 1980.
7. Newburger, A.E., Weinstein, G.D. and Young, R.C.: Kinetic analysis of combination chemotherapy for metastatic melanoma. J. Invest. Derm. 74: 259, 1980.
8. Ozols, R.F., Willson, J.K.V., Grotzinger, K.R., and Young, R.C.: Cloning of human ovarian cancer cells in soft agar from malignant effusions and peritoneal washings. Cancer Res., in press.
9. Ozols, R.F., Willson, J.K.V., and Young, R.C.: Human ovarian cancer colony formation: Growth from malignant washings and pharmacologic applications. In Salmon, S. (Ed.): Human Tumor Cloning In Vitro. New York, Alan R. Liss, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06119-11 M																																																						
PERIOD COVERED October 1, 1979 to September 30, 1980																																																								
TITLE OF PROJECT (80 characters or less) Cytogenetic Studies																																																								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																																								
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Jacqueline Whang-Peng</td> <td style="width: 20%;">Sr Investigator</td> <td style="width: 10%;">MB</td> <td style="width: 20%;">NCI</td> </tr> <tr> <td rowspan="10">Other:</td> <td>Edwin Douglass</td> <td>Clinical Associate</td> <td>MB</td> <td>NCI</td> </tr> <tr> <td>Turid Knutsen</td> <td>Med Technologist</td> <td>MB</td> <td>NCI</td> </tr> <tr> <td>Elaine Lee</td> <td>Chemist</td> <td>MB</td> <td>NCI</td> </tr> <tr> <td>Paul Bunn</td> <td>Sr Investigator</td> <td>VAMO</td> <td>NCI</td> </tr> <tr> <td>Eli Glatstein</td> <td>Chief</td> <td>RO</td> <td>NCI</td> </tr> <tr> <td>Samuel Broder</td> <td>Sr Investigator</td> <td>I</td> <td>NCI</td> </tr> <tr> <td>Michael Blaese</td> <td>Sr Investigator</td> <td>I</td> <td>NCI</td> </tr> <tr> <td>Ian Magrath</td> <td>Sr Investigator</td> <td>PO</td> <td>NCI</td> </tr> <tr> <td>Fred Li</td> <td>Sr Investigator</td> <td>E</td> <td>NCI</td> </tr> <tr> <td>Susan Seiber</td> <td>Sr Investigator</td> <td>LCHP</td> <td>NCI</td> </tr> <tr> <td>William Levis</td> <td>Sr Investigator</td> <td>D</td> <td>NCI</td> </tr> <tr> <td>Nobuyuki Shitara</td> <td>Sr Investigator</td> <td>SN</td> <td>NINCDS</td> </tr> </table>			PI:	Jacqueline Whang-Peng	Sr Investigator	MB	NCI	Other:	Edwin Douglass	Clinical Associate	MB	NCI	Turid Knutsen	Med Technologist	MB	NCI	Elaine Lee	Chemist	MB	NCI	Paul Bunn	Sr Investigator	VAMO	NCI	Eli Glatstein	Chief	RO	NCI	Samuel Broder	Sr Investigator	I	NCI	Michael Blaese	Sr Investigator	I	NCI	Ian Magrath	Sr Investigator	PO	NCI	Fred Li	Sr Investigator	E	NCI	Susan Seiber	Sr Investigator	LCHP	NCI	William Levis	Sr Investigator	D	NCI	Nobuyuki Shitara	Sr Investigator	SN	NINCDS
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COOPERATING UNITS (if any) Lab Immunology, NCI; Pediatric Oncology Br, NCI; Lab Chem Pharm, ET, NCI; Clin Pathology, ET, NCI; Radiation Oncology Br, NCI; Dermatology Br, NCI; Epidemiology Br, DCCP, NCI; BCRC, NCI; VA-MOB; Surg Neurology Br, NINCDS																																																								
LAB/BRANCH Medicine Branch																																																								
SECTION Cytogenetic Oncology																																																								
INSTITUTE AND LOCATION Clinical Oncology Program, NCI, Bethesda, Maryland 20205																																																								
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CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																																																								
SUMMARY OF WORK (200 words or less - underline keywords) The areas of investigation:																																																								
1. Cytogenetic studies of human <u>neoplastic</u>, <u>hematological</u>, and <u>congenital</u> disease. 2. In vitro <u>cytogenetic studies</u> of direct tumor material, tissue culture lines, and colony cultures derived or established from patients with <u>Burkitt's lymphoma</u>, <u>small cell carcinoma of lung</u>, and <u>cancer of the brain</u>, <u>ovary</u> or <u>testis</u>. 3. Early detection of <u>secondary leukemia</u> in patients with <u>non-Hodgkin's lymphoma</u>, <u>CLL</u>, <u>small cell carcinoma of lung</u> using <u>chromosomal abnormalities</u> as markers. 4. Detection of <u>sister chromatid exchanges</u> in families with <u>ataxia telangiectasia</u>, <u>Bloom's syndrome</u>, and a <u>high risk of cancer</u>.																																																								

5. Observation of chromosomal abnormalities in patients with cutaneous T-cell lymphomas; studies performed on peripheral blood, bone marrow, lymph node, and tissue culture lines.
6. Collaborative study of the cytogenetic and teratogenic effects of various mitotic inhibitors such as PALA and other Phase I drugs, using both in vitro and in vivo techniques.

Projects Completed in Past Year:

I. Cytogenetic study of cutaneous T-cell lymphoma (CTCL): Conclusions: CTCL progresses from an early polyclonal phase to a terminal phase with single clone proliferation. The cytogenetic findings support the impression that CTCL is a disease whose various clinical manifestations represent a chronological sequence. The cytogenetic abnormalities parallel the clinical symptoms - the more extensive the chromosome abnormalities, the more advanced the clinical disease.

II. Cytogenetic study of American Burkitt's Lymphoma: This study demonstrated that the specific translocation between chromosomes 8 and 14 is present in non-African as well as the African form of the disease. In contrast to the African Burkitt's, however, this translocation is rarely the sole karyotypic abnormality. We continue to study this chromosomal member in order to increase our understanding of the development and behavior of this tumor.

III. Cytogenetic Studies of Secondary Leukemia: A study of ANLL secondary to radiation therapy for lymphoma strongly suggests that the complex and extensive chromosome abnormalities which develop in these cases may be very useful in predicting leukemic transformation prior to the diagnosis of leukemia morphologically. Cytogenetic studies are presently being conducted in leukemia secondary to several other neoplastic diseases such as CLL and small cell carcinoma of lung, primary preleukemic conditions such as pancytopenia, and solid tumors of the brain, ovary, and testis.

IV. Studies of chromosome aberrations and sister chromatid exchanges in families with ataxia telangiectasia, Bloom's syndrome, and families with a high risk of cancer. These studies were conducted to more fully understand the mechanisms of genetic instability, and to correlate chromosome abnormalities with the early detection of malignant transformation.

V. Cytogenetic and Teratogenic Study of PALA Embryotoxicity in Mice: Conclusion: PALA-induced embryotoxicity apparently is not derived from cytogenetic damage as the incidence of chromosomal aberrations was not significantly increased by in vivo or in vitro exposure to PALA.

Publications:

1. Whang-Peng, J., Bunn, P., Knutsen, T., Schechter, G.P., Gazdar, A.G., Mathews, M.J., and Minna, J.D.: Cytogenetic abnormalities in patients with cutaneous T-cell lymphomas. *Cancer Treat. Rep.* 63: 575-580, 1979.
2. Whang-Peng, J., Knutsen, T., O'Donnell, J.R., Brereton, H.D.: Acute non-lymphocytic leukemia and acute myeloproliferative syndrome following radiation therapy for non-Hodgkin's lymphoma and chronic lymphocytic leukemia: Cytogenetic studies. *Cancer* 44: 1592-1601, 1979.

3. Ross, D.D., Wiernik, P.H., Sarin, P., and Whang-Peng, J.: Loss of terminal deoxynucleotidyl transferase (TdT) activity as a predictor of emergence of resistance to chemotherapy in a case of chronic myelogenous leukemia in blast crisis. *Cancer* 44: 1566-1570, 1979.
4. Whang-Peng, J., Lee, E.C., and Hubbell, R.F.: Karyology. In Altman, P. L. and Katz, D.D. (Eds.): Inbred and Genetically Defined Strains of Laboratory Animals, Part 2: Hamster, Guinea Pig, Rabbit, and Chicken. Bethesda, Maryland, FASEB, 1979, pp. 515-520.
5. Douglass, E.C., Magrath, I.T., Lee, E.C., and Whang-Peng, J.: Cytogenetic studies in non-African Burkitt's lymphoma. *Blood* 5: 148-155, 1980.
6. Whang-Peng, J and Knutsen, T.: Lymphocytic leukaemias, acute and chronic. In Penington, D.C. (Ed.): Clinics in Haematology, 1980, Vol.9, pp.87-127.
7. Magrath, I.T., Pizzo, P.S., Whang-Peng, J., Douglass, E.C., Alabaster, O., Gerber, P., Freeman, C.B., and Novikovs, L.: Characterization of lymphoma-derived cell lines: Comparison of cell lines positive and negative for Epstein-Barr virus nuclear antigen. I. Physical, cytogenetic, and growth characteristics. *JNCI* 64: 465-476, 1980.
8. Bunn, P.A., Whang-Peng, J., Carney, D.N., Schlam, M.L., Knutsen, T., and Gazdar, A.F.: DNA content analysis by flow cytometry and cytogenetic analysis in mycosis fungoides and Sezary Syndrome: Diagnostic and prognostic implications. *J. Clin. Invest.*, in press.
9. Douglass, E.C., Magrath, I.T., Lee, E.C., and Whang-Peng, J.: Serial cytogenetic studies of non-endemic Burkitt's lymphoma cell lines. *JNCI*, in press.
10. Seiber, S.M., Botkin, C.C., Soong, P., Lee, E.C., Whang-Peng, J.: Embryotoxicity in mice of phosphonacetyl-L-aspartic acid (PALA), a new antitumor agent. I. Embryo-lethal, teratogenic, and cytogenetic effects. *Teratology*, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06700-07 M																														
PERIOD COVERED October 1, 1979 to September, 1980																																
TITLE OF PROJECT (80 characters or less) Clinical Program in Breast Carcinoma																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Marc E. Lippman</td> <td style="width: 20%;">Sr Investigator</td> <td style="width: 10%;">M</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>Jane Cassidy</td> <td>Nurse</td> <td>M</td> <td>NCI</td> </tr> <tr> <td></td> <td>Richard Simon</td> <td>Chief</td> <td>BR</td> <td>NCI</td> </tr> <tr> <td></td> <td>Allan Lictor</td> <td>Sr Investigator</td> <td>RO</td> <td>NCI</td> </tr> <tr> <td></td> <td>Ernest DeMoss</td> <td>Sr Investigator</td> <td>S</td> <td>NCI</td> </tr> <tr> <td></td> <td>Sandra Levy</td> <td>Sr Investigator</td> <td>DCCR</td> <td>NCI</td> </tr> </table>			PI:	Marc E. Lippman	Sr Investigator	M	NCI	Other:	Jane Cassidy	Nurse	M	NCI		Richard Simon	Chief	BR	NCI		Allan Lictor	Sr Investigator	RO	NCI		Ernest DeMoss	Sr Investigator	S	NCI		Sandra Levy	Sr Investigator	DCCR	NCI
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COOPERATING UNITS (if any) Biometrics Research Branch, NCI; Radiation Oncology Branch, NCI; Surgery Branch, NCI																																
LAB/BRANCH Medicine Branch and Division of Cancer Control and Rehabilitation																																
SECTION Medical Breast Cancer Section																																
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SUMMARY OF WORK (200 words or less - underline keywords) The Medical Breast Cancer Section is responsible for the development of a clinical and laboratory program directed at <u>breast cancer</u>. <u>Clinical trials in metastatic disease</u> comparing <u>chemotherapeutic</u>, <u>hormonal</u> and <u>chemohormonal</u> regimens are underway. Biochemical and hormonal <u>marker studies</u> are undertaken and coordinated by the Medical Breast Cancer Section. These studies consist of a major chemotherapy trial aimed at synchronizing human breast cancer cells with hormonal agents for more successful cell cycle phase specific chemotherapy; a hormonal therapy trial aimed at prospectively evaluating the usefulness of steroid receptors for estrogens, androgens and progestins in human breast cancer; an advanced disease hormonal therapy trial comparing tamoxifen to tamoxifen plus an androgen, several phase II trials including a trial of PALA and 5FU in combination and 13 Cis Retinoic acid. In addition there is an endocrine and chemotherapy program for male breast cancer. A cooperative trial between the																																

Surgery, Radiation and Medicine Branches is underway comparing excisional biopsy plus definitive radiotherapy to simple mastectomy in clinical Stage I and II breast cancer. All patients have axillary dissections; A-C chemotherapy is given to all N+ patients.

Finally, a prospective psychological study aimed at discovery whether or not patients' emotional response to their disease influences outcome is underway.

Project Description:

The Medical Breast Cancer Service was established in July 1972 and the clinical program was initiated in January 1973. It was responsible to the Office of the Associate Director, COP, until its shift to the Medicine Branch in August 1974.

I. Clinical Trials

A. Recurrent disease trials.

1. MB-160, a randomized trial of chemotherapy + hormonal therapy aimed at inducing cell synchrony was initiated in August of 1976. This trial has patients on study and is too early for evaluation although preliminary analysis suggests ER+ patients may benefit from synchronization.
2. MB151, a randomized trial of hormonal therapy in first recurrent breast cancer in which steroid hormone receptors are to be evaluated prospectively. Twenty-eight patients were entered. This study has been closed due to inadequate accrual.
3. MB132, a randomized trial of tamoxifen + halotestin. This trial, nearing completion (112 patients entered), has suggested preliminarily that tamoxifen plus halotestin is superior in response rate and that both regimens are substantially less effective in heavily pre-treated patients.
4. A Phase II trial of CIS platinum in breast cancer is completed. There were no significant PR's or CR's in this trial of 22 patients.
5. A Phase II trial of chlorozotcin has been closed. Overall response rate suggests little activity for this agent in heavily pretreated patients.
6. A Phase II trial of PALA-5FU in combination is underway. This trial is too early for analysis but several responses have been seen.
7. A Phase II trial of 13 cis retinoic acid in breast, ovary, testicular cancers and melanoma is underway.

8. A psychological study of how patient attitudes influence survival is ongoing.
9. A protocol for sequential endocrine approaches to male breast cancer with concurrent receptor analyses is ongoing.

II. Ancillary Studies

A. Steroid Binding Proteins (SBP)

SBP are being prospectively evaluated in all breast cancer samples. These include analyses for androgen, estrogen, glucocorticoid and progesterin receptors. In addition, analyses are being performed on melanoma, ovary, colon, male breast and hematologic malignancies. Studies of retinoic acid and retinol binding proteins in breast cancer are also being carried out. Numerous publications resulting from these data are listed below.

III. Extramural Activities

A. Breast Cancer Task Force.

Dr. Lippman is on the Steering Committee of the Breast Cancer Task Force.

B. National Surgical Adjuvant Breast Project

Dr. Lippman is on the Endocrine Committee of the National Surgical Adjuvant Breast Project.

C. Outside Teaching Responsibilities.

Dr. Lippman is Associate Clinical Professor of Medicine and Pharmacology at the USUHS Medical School

Publications:

1. Lippman, M.E., Konior Yarbrow, G., and Leventhal, B.G.: Clinical correlations of glucocorticoid receptors in human leukemia in steroid receptors and the management of cancer by E.B. Thompson and M.E. Lippman, CRC Press, Vol 1, 5:68-80, 1979.
2. Allegra, J.C., Lippman, M.E., Thompson, E.B. and Simon, R.: The influence of steroid hormone receptors in response to chemotherapy in patients with metastatic breast cancer in steroid receptors and the management of cancer. E.B. Thompson and M.E. Lippman (Eds). CRC Press, Vol 1, 4: 58-66, 1979.
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4. Everson, R.B., Lippman, M.E., Thompson, E.B., McGuire, W.L., DeSombre, E.R., Wittliff, J.L., Singhakowinta, A., and Neifeld, M.P.: Steroid receptors in male breast cancer. *Cancer Research* 40: 991-997, 1980.
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6. Allegra, J.C., Lippman, M.E., Thompson, E.B., Simon, R., Barlock, A., Green, L., Huff, K., Do, H.M.T., Aitken, S., and Warren, R.: The relationship between the progesterone, androgen and glucocorticoid receptor and the response rate to endocrine therapy in metastatic breast cancer. *Cancer Research* 39: 1973-1979, 1979.
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8. Allegra, J.C. Lippman, M.E., Green, L., Barlock, A., Simon, R., Thompson, E.R., Huff, K.K., and Griffin, W.: Estrogen receptor values in patients with benign breast disease. *Cancer* 44: 228-231, 1979.
9. Everson, R.B. and Lippman, M.E.: Male breast cancer in Breast Cancer: Advances in Research and Treatment, Vol III. W.L. McGuire (ed). Plenum Press, N.Y., 239, 267, 1979.
10. Lipsett, M.D. and Lippman, M.E.: Endocrine responsive cancers of man. In Endocrinology. R.H. Williams (Ed). W.B. Saunders, Philadelphia, 1979, in press.
11. Allegra, J.C., Lippman, M.E., Thompson, E.R., Simon, R., Barlock, A., Green, L., Huff, K.K., Do, H.M.T., Aitken, S., and Warren, R.: The relationship between steroid receptor status and disease free interval in breast cancer. *Cancer Treatment Reports* 63: 1271-1277, 1979.
12. Lippman, M.E.: Principles of hormone therapy. In Cancer Chemotherapy. Carter, S. and Glatstein, E. (Eds). Academic Press, N.Y. In press.
13. Allegra, J.C., Simon, R., and Lippman, M.E.: The association between steroid hormone receptor status and the disease free interval in breast cancer. In Adjuvant Therapy of Cancer. Jones, W.E. and Salmon, S. (Eds) Grune and Stratton, New York, 1979, 47-54, 1979.
14. Allegra, J.C. and Lippman, M.E.: Estrogen receptor determination predicts response to tamoxifen therapy. In A New Approach to the Endocrine Treatment of Breast Cancer. Springer-Verlag, Berlin. In press.

15. Lippman, M.E.: Interpreting steroid receptor assays. *Western J. Med.* 130: 450-451, 1979.
16. Lippman, M.E. and Allegra, J.C.: Quantitative estrogen receptor analysis, the response to endocrine and cytotoxic chemotherapy in human breast cancer and the disease free interval. *Cancer*. In press.
17. DeWys, W., Allegra, J.C., Simon, R., and Lippman, M.E.: Prediction of response to endocrine therapy in breast cancer from the estrogen receptor status of one site and the number of metastatic sites: A hypothesis. *Cancer Research*. In press.
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06702-05 M
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Mechanisms of Hormone Dependence of Human Malignancy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Marc E. Lippman Other: Marie E. Monaco Ming Ta Chong Diane Bronzert Kenneth Cowan Andre Lacroix Lawrence Tamarkin Bruce Chabner	Sr Investigator Cancer Expert Clinical Associate Technician Clinical Associate Guest Worker Sr Investigator Chief	M M M M M M IRP CP NCI NCI NCI NCI NCI NCI NCI NCI NCI
COOPERATING UNITS (if any) Laboratory of Biochemistry, NCI; Endocrinology Branch, NICHD; Clinical Pharmacology Branch, NCI		
LAB/BRANCH Medicine Branch		
SECTION Medical Breast Cancer Section		
INSTITUTE AND LOCATION Clinical Oncology Program, NCI, Bethesda, Maryland 20205		
TOTAL MANYEARS: 10	PROFESSIONAL: 10	OTHER:
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) We are investigating the mechanisms whereby <u>steroid</u> and <u>polypeptide hormones</u> stimulate growth and specific protein synthesis in human breast cancer both in <u>tissue culture</u> model systems we have established and in clinical settings. A. We are studying the prevalency and clinical correlates of specific <u>steroid receptors</u> for <u>estrogen</u> , <u>androgen</u> , <u>glucocorticoid</u> and <u>progesterone</u> in <u>human breast cancer</u> , <u>lymphomatous diseases</u> , <u>melanoma</u> , <u>colon carcinoma</u> , <u>ovarian cancer</u> and <u>male breast cancer</u> . B. Assays for specific gene products (<u>α-lactalbumin</u> , <u>casein</u> , <u>fatty acid</u> , <u>sythetase</u> , <u>thymidine kinase</u>) have been developed and we are studying the effects of steroid <u>hormones</u> , <u>insulin</u> and <u>vasopressin</u> on the activities and synthesis of these proteins.		

- C. We are investigating glucocorticoid receptors in various subpopulations of normal and leukemic lymphoid cells, including their regulation by mitogens such as phytohemagglutinin. In addition we are studying glucocorticoid receptors in Burkitt's Lymphoma, hairy cells leukemia, ANLL and lymphomas.
- D. We have developed a vasopressin responsive rat mammary cancer cell line in tissue culture. This is the first in vitro long term vasopressin dependent system to be characterized and as such, appears particularly promising for the study of the mechanism of vasopressin action.
- E. We have developed a completely defined hormone supplemented medium (T3, insulin, transferrin and estradiol) which supports the growth of human mammary cancer in long term tissue culture.
- F. We are characterizing and purifying the human estrogen receptor from breast cancer cell lines. We have currently achieved a 1400 fold purification using affinity chromatography.
- G. We are studying intracellular pharmacokinetics of estrogen and antiestrogen metabolism and efflux from human breast cancer cells using perfusion systems.
- H. We are studying the detailed regulation of DNA synthesis in human breast cancer cells and as such have developed ways for accurately quantifying total DNA synthesis together with the scavenger and denovo pathways of pyrimidine biosynthesis.
- I. We are studying fluropyrimidine methotrexate interactions in human breast cancer cells maintained in defined, serum free nucleoside medium and as such have worked out optimal scheduling of drugs for enhanced cytotoxicity.
- J. We are studying the effects of retinoids on human breast cancer cell lines in vitro and have developed means of quantifying separable retinoic acid and retinol binding proteins.
- K. We have developed a soft agar cloning technique which have permitted the development of clones of antiestrogen resistant variant (putative mutant) cell lines derived from hormone dependent wild typed cells. These variant cells are currently being analyzed biochemically and via somatic cell hybridization.
- L. We have developed methotrexate and PALA resistant cell lines from human breast cancer cells. Pathways of resistance are under investigation.
- M. We have developed assays for all of the enzymes in the denovo pyrimidine biosynthetic pathway and their hormonal regulation is under investigation.

- N. The effects of pineal function and its secretion melatonin are being explored as modulators of breast cell growth in vivo and in vitro.
- O. We are studying the interactions of novel ligands with human estrogen receptor. The goal is to develop better assays and evaluate cytotoxics linked to the hormone moiety. These have included 16 α -iodo estradiol and estracnu (estradiol plus a nitrosourea).

Publications

1. Strobl, J.S. and Lippman, M.E.: Long term nuclear retention of estradiol by human breast cancer cells in tissue culture. *Cancer Research* 39:3319-3327, 1979.
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3. Golde, D.W., Bersch, N., and Lippman, M.E.: Dexamethasone inhibition of Friend leukemia cell proliferation and differentiation in mediated via glucocorticoid receptor. *Proc. National Acad. Science*, Vol 76, 3315-3317, 1979.
4. Donehower, R., Allegra, J.C., Lippman, M.E. and Chabner, R.: Sequence dependent effects of methotrexate and 5-fluoropyrimidine in serum free cultures of human breast cancer. *European Journal of Cancer*, in press.
5. Allegra, J.C. and Lippman, M.E.: The effects of 17 estradiol and tamoxifen on the ZR-75-1 human breast cancer cell line in defined medium. *Eur. Journal of Cancer*, in press.
6. Koeffler, H.P., Golde, D.W. and Lippman, M.E.: Glucocorticoid sensitivity and receptors in cells of human myelogenous leukemia lines. *Cancer Res* 40: 563-566, 1980.
7. Lacroix, A. and Lippman, M.E.: Binding of retinoids to human breast cancer cell lines and their effects on cell growth. *J. Clin. Invest.* 65: 586-591, 1980.
8. Chong, M. and Lippman, M.E.: Purification of estrogen receptors from MCF-7 human breast cancer cells. *Cancer Res.*, (in press).
9. Eil, C., Lippman, M.E. and Loriaux, D.L.: A dispersed-whole cell method for the determination of androgen receptors in human skin fibroblasts. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06708-01 M
PERIOD COVERED October 1, 1979 to September, 1980		
TITLE OF PROJECT (80 characters or less) Genetic Regulation of the Immune Response		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Dan L. Longo Other: William Paul Ronald Schwartz Patricia Dobson	Sr Investigator Chief Sr Investigator Technologist	M LI LI M NCI NIAID NIAID NCI
COOPERATING UNITS (if any) Laboratory of Immunology, NIAID		
LAB/BRANCH Medicine Branch		
SECTION		
INSTITUTE AND LOCATION Clinical Oncology Program, NCI, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) 1. Analysis of the <u>expression</u> of <u>immune response genes</u> in T cells, B cells, and antigen presenting cells. 2. Study of the <u>mechanism of action</u> of the <u>thymus</u> on the development of T cell repertoire for antigen recognition and self-recognition. 3. Experiments on the <u>ontogeny</u> of <u>antigen presenting cells</u> and their <u>turnover</u> in spleen, peritoneal cavity, and thymus.		
Selected Highlights of Work Completed This Year: 1. <u>Gene Complementation</u> : We have demonstrated that the genes which control the responsiveness to a given antigen need not be present in the genome of the responding T cell as long as it has matured in an environment in which the gene was present (Longo & Schwartz:J.Exp.Med., in press). We have also demonstrated that		

the mechanism by which a genetic nonresponder T cell "learns" to respond to a given antigen is by developing a receptor for the antigen in conjunction with immune response gene products on the surface of the antigen presenting cell, i.e., the T cell sees both structures, antigen and Ir gene products (Longo & Schwartz: Proc.Natl.Acad.Sci, in press).

We have further shown that the alteration in the T cell receptor repertoire which occurs in the thymus may be caused by antigen presenting cells in the thymus. These thymic antigen presenting cells may determine what the T cell emerging from the thymus is capable of doing (Longo & Schwartz: Nature, in press).

Further studies have examined the cellular requirements for mounting an antibody response to T-independent antigens, of which the pneumococcal polysaccharide vaccine is an example (Kirkland et al: J. Immunol.).

Publications:

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3. Longo, D.L. and Schwartz, R.H.: Gene complementation: Neither Ir-GL $\emptyset$ need be in the proliferative T cell to generate an immune response to GL $\emptyset$. *J. Exp. Med.*, in press.
4. Longo, D.L. and Schwartz, R.H.: Chimeric T cells become phenotypic responders by acquiring the ability to recognize responder Ia antigens as self. *Proc. Natl. Acad. Sci., U.S.A.*, in press.
5. Longo, D.L. and Schwartz, R.H.: T cell specificity for H-2 and Ir gene phenotype correlates with the genotype of thymic antigen-presenting cells. *Nature*, in press.
6. Longo, D.L., Matis, L.A., and Schwartz, R.H.: Insights into immune response gene function from experiments with chimeric animals. *CRC Crit. Rev. Immunol.*, in press.

October 1, 1979 to September 30, 1980

ANNUAL REPORT OF THE NCI-VA MEDICAL ONCOLOGY BRANCH
OF THE DIVISION OF CANCER TREATMENT, NATIONAL CANCER INSTITUTE
AND THE WASHINGTON, DC VETERANS ADMINISTRATION MEDICAL CENTER

1.0 Summary

1.1 Description

The NCI-VA Medical Oncology Branch is an intramural branch of the COP/DCT/NCI/NIH, a section of the Medical Service and a section of the Research and Education (R & E) Service of the Washington, D.C. Veterans Administration Medical Center (WVAMC) and thus is designated as a Program by the WVAMC and the Veterans Administration Central Office (VACO). The primary goals of the NCI-VA Medical Oncology Branch are: investigation of patients with malignant disease in clinical therapeutic trials; laboratory investigations of tumor cell biopsy. Secondary aims are to provide a service function to the WVAMC for management of patients with malignant disease and training of fellows in clinical oncology and investigation

1.2 Administration

The NCI-VA Medical Oncology Branch is a cooperative program between the COP/DCT/NCI/NIH and the VA via an interagency agreement. The NCI provides for a transfer of funds and several senior staff personnel and administrators from the CT/NCI to VACO and WVAMC. The WVAMC provides inpatient ward (2CN), office, outpatient clinic, and research laboratory space, billets to run the program, clinical laboratory, radiology and pharmacy services as well as subspecialty medical and surgical consultations for patients. Both veteran and nonveteran patients are entered into clinical protocols approved by clinical review committees of the WVAMC and the NCI. A yearly contract is signed between the VACO and the COP/DCT covering the interagency agreement and project plan. The NCI reimburses the WVAMC for non-veteran patients through a set of fixed daily inpatient and outpatient charges and pays an overhead charge for administrative and other support to the WVAMC. The WVAMC provides for some financial coverage for patient care.

1.3 Clinical Resources

All clinical and ward supporting work for protocol and non-protocol patients is performed by four senior staff oncologists, one infectious disease specialist, two pathologists, six first year, five second and third year clinical associates, two rotating interns, one pharmacist, three research nurses, 22 full time and part time nurses, two medical technologists, three ward and outpatient clinic secretaries and one medical DMT. WVAMC provides part time use of a dietitian, food service workers, social worker, housekeeping aid and ward supply. The inpatient unit has 30 beds and an outpatient treatment area. The outpatient clinic runs three half days a week in the WVAMC outpatient area, and five days a week in the inpatient (2CN) ward area.

A satellite ambulatory care program (SACP) with five rooms in the United Inn, Bethesda, Md. runs as part of the COP/DCT outpatient facility contract with NCI funding. A driver and a van (funded by the NCI transferred funds) transports patients to and from the SACP and the main NIH campus for various procedures. Radiotherapy is primarily handled by the Radiation Oncology Branch (ROB/DCT/NCI) at the NIH campus. Anti-neoplastic drugs are provided by the NCI. All transportation costs for protocol patients are met by the NCI transferred funds.

1.4 Clinical Patient Load

The NCI-VA Medical Oncology Branch assumes complete primary care responsibility (including inpatient and outpatient) for all patients on its protocols and in addition, the primary care for veteran patients deemed likely by the NCI-VA M.O.B. Chief to have significant benefit from complex chemotherapy treatment regimens. This amounts to approximately 300 patients per year. The program also provides for consultative services for all patients with malignant disease as requested in the inpatient and outpatient areas (approximately 40 consultations per month). The program provides for outpatient chemotherapy and care for WVAMC veterans requiring non-protocol chemotherapy (approximately 100 patients in FY 1980). The inpatient ward (2CN) has had approximately 80% occupancy, and there are approximately 240 outpatient visits per month. Non-veteran inpatient occupancy has been approximately 30% of the total, and non-veteran outpatient visits approximately 20% of the total. A number of the veteran patients come for protocol treatment from outside the WVAMC area and the WVAMC hospital district on NCI transferred funds.

The senior staff provides 6 months of inpatient attending coverage on the general medical service, two months of attending coverage on the Infectious Disease Section consultation service; provides consultation when asked by the WVAMC tumor registry; participates in the WVAMC Cancer Committee and weekly Medical-Surgical Chest Conferences; provides weekly consult rounds conference for patients seen in consultation, and multiple individual conferences for the WVAMC through the general hospital education program. Also, the NCI-VA pathologists provided additional opinions on histologic material to the hospital.

1.5 Oncology Fellowship Training Program

As part of the oncology fellowship training program of the Clinical Oncology Program (COP)/DCT/NCI/NIH the NCI-VA Medical Oncology Branch provides for clinical training in the subspecialty of Medical Oncology for the Clinical Associates assigned to it (as special positions designated by the VACO) or to the USPHS officer rotating from the Clinical Center, NIH. In addition, it provides general medical and oncology training to twelve interns from the Medical Service, WVAMC, George Washington University and Georgetown University that rotate through the 2CN inpatient ward.

1.6 Laboratory Research Facilities and Staffing

The laboratory research program in tumor cell biopsy is carried on in 3000 square feet of wet lab space. The program has the use of common research space of the WVAMC R & E Service including instrument storage and animal rooms totaling an additional 1500 square feet of space. There are four senior investigators, two post doctoral fellows, four clinical associates, and 10 technicians working in this area.

1.7 Major New Administrative Developments

The plans for the move of the NCI-VA Medical Oncology Branch to the National Naval Medical Center in Bethesda, Maryland continue. The architectural plans for the NNMC facility were completed by the NCI-VA staff. The proposed contract governing the operating of the unit at the NNMC was also completed by the NCI-VA staff and forwarded to the NNMC for the final decision. As requested by the Chief Medicine, WVAMC, a list of various personnel decisions governing the laboratory staff was given to the hospital. The WVAMC has established a committee to make plans for a new Oncology Unit when the present unit moves to the NNMC. The current Unit has provided any requested information to the hospital. The NCI-VA Staff have been in discussions with the NNMC staff about interactions in clinical oncology and plan to start protocol planning discussions. Ms. M. A. Anerino has begun work as the new Administrative Assistant for the NCI-VA Branch. Because of the rise in salaries, the high rate of inflation adding to the cost of supplies, services, equipment and patient travel, significant cutbacks had to be made in various portions of the clinical and laboratory programs to stay within a level budget. This produced a more focused effort in research work.

1.8 Statistical interaction and Data Collection

The NCI-VA Branch has been particularly fortunate to have the collaboration of Dr. Robert Makuch of the Biometry Branch/NCI in the conduct of our clinical trials. This includes both randomized and non-randomized trials, and also retrospective reviews of patient data. We want to involve Dr. Makuch to the fullest possible extent in the planning of the next generation of protocols and to this end Dr. Simon has appointed him as permanent statistician to our branch. Dr. Makuch will play a big role in this regard in the development of protocols with the National Naval Medical Center and the implementation of the CAPRI system for data collection designed by the Biometry Branch.

1.3 Summary of Clinical Investigations

Diseases studied in detail by the NCI-VA Medical Oncology Branch include: lung cancer (small cell, adenocarcinoma, large cell carcinoma, and epidermoid cancer); prostate carcinoma; cutaneous T-cell lymphomas (Mycosis fungoides and the Sezary syndrome); multiple myeloma; and hepatocellular carcinoma.

A major treatment study focuses on small cell bronchogenic carcinoma (SCCL). In limited stage SCCL a prospective randomized trial of intensive combination chemotherapy with or without simultaneous chest radiotherapy (CMC + Chest RT + VAP; NCI-VA Protocol 77-4) is being conducted in collaboration with the Radiation Oncology Branch/DCT. This study asks whether the two modalities given simultaneously are better than chemotherapy alone with respect to complete response rate, long term disease free survival, median survival and toxicity (acute and chronic). Thirty two patients have been entered, 16 on each arm. The initial complete response rate, local relapse rate, and long term disease free survival all currently favor combined modality therapy but the differences are not statistically significant. There were three early deaths in the combined modality arm secondary to acute diffuse interstitial pulmonary disease, as well as two episodes of severe esophagitis. This has necessitated reshaping radiotherapy fields three times during therapy and meticulous attention to radiotherapeutic detail. With these technical additions, the toxicity of combined modality has not been bothersome. We plan to continue this very important trial until there are clear statistical differences between the two treatments or until 25-30 patients have been entered on each arm.

In extensive stage SCCL we have tested a new combination (cyclophosphamide, adriamycin, VP-16-213, and vincristine; "CAPO", NCI-VA Protocol 77-3) given in a large number of cycles during induction therapy. The purpose of this protocol was to test the effect of giving a large number of high dose, combination chemotherapy cycles during 6-8 weeks of induction therapy and to compare the results to our prior CMC-VAP trial in extensive stage SCCL of one intense course of therapy followed by a moderate dose therapy with a non-cross resistant regimen. 50 patients entered the study between November 1977, and November 1979 when the study closed, and received different numbers of induction cycles according to time of protocol entry. 34 patients were treated with 3-6 cycles (11 received 5-6 cycles, and 23 3-4 cycles) for a mean of 4.5 cycles of induction; while 16 patients were treated with only 2 cycles of induction therapy. The decrease in the number of cycles was necessitated by the toxicity of the most intense regimens. The complete response rate for the 3-4 cycle group was 26% and the objective tumor response rate 100% compared to a 13% CR rate for the 2 cycle group and 63% overall tumor response rate. The prior CMC-VAP trial had a 36% CR rate and 91% tumor response rate. The actuarial survival curves, and disease free survival curves for all of the groups were not significantly different. However, the 3-4 cycle group had 18% induction deaths compared to 6-7% for the 2 cycle or CMC-VAP induction treatments. This treatment represented the most intense treatment ever given to extensive stage SCCL and produced a clear answer: Increasing the number of intense induction cycles in extensive stage SCCL did not increase the CR rate compared to other therapies, but did increase the treatment

related toxicity and mortality. Again a steep dose response curve was seen with moderate dose (2 Cycle) compared to more intense (3-6 cycle) therapy as seen in our prior trials.

We conclude from these studies that other methods besides increasing the initial intensity of induction therapy, either by dose or number of induction cycles of the same combination, or by alternating non-cross resistant combinations need to be considered in the treatment of extensive stage SCCL.

Thus, to summarize the results of prior trials in SCCL performed by the NCI-VA Oncology Branch we feel: that: 1.) high dose combination chemotherapy produces a high complete response rate, increased survival and the first long term cured survivors (NCI-VA Protocol 73-4); 2.) that adding non-cross resistant combinations increases the complete response rate but that these complete responses are less durable than ones achieved with the initial therapy and thus survival is not significantly affected (NCI-VA Protocol 75-4); 3.) that increasing the cyclophosphamide dose does not increase the complete response rate or survival (NCI-VA Protocols 73-4, 75-4, 77-1, 77-3); 4.) that adding chest radiotherapy after initial CMC-VAP chemotherapy does not increase the complete response rate or survival (NCI-VA Protocol 77-1); 5.) that increasing the number of induction cycles does not increase the complete response rate or survival (NCI-VA Protocol 77-3); 6.) that simultaneously combining chemotherapy and chest radiotherapy may increase the complete response rate and disease free survival (NCI-VA Protocol 77-4). Thus, for extensive stage disease we felt a radical new departure was necessary. This led to the design of NCI-VA Protocol 80-3: Intensive combined modality (chemotherapy plus multiple field local irradiation therapy followed by autologous bone marrow hematopoietic reconstitution for patients with extensive stage SCCL in complete or partial remission in collaboration with the Radiation Oncology, Pediatric Oncology, and Biometric research branches.

Many extensive stage patients with SCCL initially present with symptoms related to tumor bulk. The initial therapy should "debulk" their tumor and allow return of a maximum possible performance status. Results with acute leukemia in the initial remission using a early intensification program with total body irradiation and chemotherapy followed by sibling allogeneic transplants have been exciting with long term disease free survival. We are trying to apply the same concept to SCCL. However because of the age and chronic cardiopulmonary status of many of these patients we feel that initial use of high dose total body irradiation may be too much of a risk.

In addition, analysis of relapse, suggest that radiotherapy in high doses may be needed for control of sites of initial bulk disease. However, we know from pilot studies of the chemotherapy alone that 35-45 days of severe marrow suppression will be anticipated. Thus because of the addition of high dose local field irradiation we feel that it is important to reduce the potential risks of such treatment by the autologous bone marrow replacement. This treatment involves standard CMC-VAP induction combination chemotherapy. At week 13, patients are restaged, and autologous bone marrow is collected and cryopreserved (if free of tumor cells histologically and by the clonogenic assay). Multiple field local irradiation in 5 days followed by intensive new chemotherapy for 3 days (Cyclophosphamide, and VP-16-213)

is given, followed in turn by autologous bone marrow replacement to aid in blood count recovery.

After recovery of hematopoietic function, patients in a complete response will be followed without further antineoplastic therapy except for prophylactic cranial irradiation.

We plan to initially pilot 15-30 patients through this regimen for toxicity studies as well as to compare them to our historical controls with extensive stage disease (75 patients treated with intensive but conventional combination chemotherapy). This should allow detection of a three-fold increase in 2-year (long term) survival compared to the historical controls. If the early results and toxicity studies are positive we would plan a randomized comparison to standard intensive treatment.

Because of non-availability of calf fraction V thymosin from Hoffman-LaRoche we have been unable to continue to randomize patients to receive or not receive initial thymosin treatment. However, a total of 67 patients had been randomized to receive thymosin 60, 20, or 0 mg/M² during the initial 6 weeks of therapy. (NCI-VA Protocols 76-4, 77-1, 77-3, 77-4). Long term (over 2.5 year) followup of these patients continues to reveal a survival advantage for patients treated with thymosin 60 compared to the other groups. Because thymosin therapy may be a clinically significant advance we have turned to other sources of thymosin. This has led to the phase I trials of human thymic hormone - like activity (Syntex Research Product SR-270258) in lung cancer (NCI-VA protocol 79-5). We are trying to determine not only toxicity but also possible effects on lymphocyte function in patients in collaboration with the Laboratory of immunodiagnosis (Dr. R. Herberman, NCI).

We have several phase II trials in patients with SCCL who have progressed on their primary multi-drug therapy regimens. These include a phase II trial of cis-platinum (NCI-VA protocol 77-7). We note that several major institutions have incorporated this drug into their first line treatment of SCCL. However, we have not been able to document significant clinical activity of this drug in relapsed patients. Other trials include: a phase II trial of hemibody radiotherapy with and without radioprotector WR-2721 (NCI-VA Protocol 77-2), in collaboration with the Radiation Oncology Branch. Previously we had found that fractionated hemibody radiotherapy (a potentially useful "systemic" therapy) had significant hematologic toxicity in previously treated patients. Thus, we have added the radioprotector to see if this toxicity could be ameliorated. The NCI-VA Branch is the only group that has published on the lack of efficacy of local radiotherapy in controlling tumor after relapse from chemotherapy alone, despite the supposed radiosensitivity of SCCL. We are thus testing the use of high dose local radiotherapy in this respect in collaboration with the Radiation Oncology Branch.

In addition to trying to increase the quality and quantity of life of the majority of patients treated the NCI trials in SCCL have had as a major goal the cure of this tumor. In collaboration with the Radiation Oncology Branch we recently reported on the potential cure of up to 12% of all patients entering NCI trials on the NCI-VA and ROB branches from March, 1973 to July

1977 to allow sufficient time for long term followup. The purpose of this analysis was to document the possibility of cure, not generally recognized, and to define important predictive and therapeutic factors in achieving this state. The long term survivors were defined as patients alive and free of disease 30 or more months from the start of therapy. The 20 long term survivors, now have a median disease free survival approaching 4 years with a range of 3 to 6 years. In fact currently 25% of all limited stage and 3% of all extensive stage SCCL patients achieve 30 month + disease free survival. We have found that important factors include initial limited stage disease; good performance status; and the use of combined modality therapy, and the early achievement of a complete response. We have also found that the duration of chemotherapy in achieving cure may be as short as 4 months. All of the patients have entered and remained in an initial systemic complete response and only 1 of the 20 patients free of disease at 30 months has relapsed despite the long time off of all therapy (18+ - 60+ months). There have however, been long term toxicities including acute leukemia in one patient and pulmonary fibrosis in 7 patients. Our future trials are directed at the role of combined modality therapy, increasing the initial complete remission rate; and the reduction in both early and late treatment related complications so as to increase the number of long term, disease free, survivors with good quality of life.

The senior staff have completed several reviews, and book chapters dealing with therapeutic results, staging procedures and prognostic factors of SCCL, as well as presentation of these overviews at the national ASCO meeting.

Non-small cell lung cancer (epidermoid, adenocarcinoma, and large cell carcinoma) accounts for over 75,000 new cancer patients annually in the USA and thus is a major health problem. The major problems in treating these cancers are: 1.) the large majority of cases have distant metastases not allowing for curative local modalities such as surgery and radiotherapy; and 2.) they are much less sensitive to chemotherapy than is SCCL. Because of these features we are conducting an intensive laboratory investigation of all histologic types of lung cancer including small and non-small cell carcinomas with a particular emphasis on in vitro drug sensitivity testing. We are also conducting standard phase II trials such as NCI-VA Protocol 78-4, a Phase II trial of doxorubicin, and Mitomycin-C in advanced non-small cell lung cancer. This regimen has given over a 50% response rate in adenocarcinoma of the lung but no responses in the other non-small cell types. However, there have been significant treatment related complications with the three week cycle regimen of both drugs particularly the development of interstitial pneumonitis in 5 of 22 patients (2 severe). Thus, we have switched the Mitomycin -C to every 6 weeks and have not seen lung toxicity, (conventional scheduling). We plan to develop a new phase II trial based on selecting drug therapy on the results of in vitro sensitivity testing in the human tumor colony forming assay (See laboratory summary).

Gastric carcinoma is a GI malignancy that is responsive to chemotherapy. One of the major problems is finding agents other than 5-FU, adriamycin, mitomycin-C and the nitrosoureas which gastric cancer is sensitive to.

We are completing a phase II trial of ICRF-159 (NCI-VA Protocol 77-17) a drug with supposed activity against other GI malignancies. However, we have only seen 2 responses in 18 patients. This is the only phase II trial in gastric cancer with this agent so it will be important to complete. We plan to use the in vitro tumor colony forming assay on material obtained at laparotomy in collaboration with the Radiation Oncology Branch, and the Georgetown Division of Oncology to select for chemotherapy that will be used in the time of relapse from their primary protocols in our future studies.

A major project of the NCI-VA Branch are the clinical treatment studies in the cutaneous T-cell lymphomas (Mycosis fungoides and the Sezary Syndrome (MF-SS)). While these are uncommon diseases (10,000 or less in the USA each year) they are of very great biologic interest. First, they permit and stimulate prospective randomized trials of conservative (palliative) versus aggressive therapy with curative intent. In this regard their biology and natural history are very similar to the nodular B cell lymphomas. Second, they allow testing of combined modality chemotherapy and radiotherapy. Third, they provide material for cell biologic studies. These diseases represent malignant expansions of clonal subsets of human T-cells for study of immunologic function and membrane properties. CTCL patients could very well be producing their own T cell growth factor (TCGF) as we have shown that some of their cells cultured in vitro produce this TCGF. If this is the case, these patients may become the prime source for this very important growth factor which is currently being used to try to grow human T cells that would be of immunotherapeutic interest. In addition, in collaboration with Dr. Gallo's laboratory we have started a cell line which has turned out to be a constitutive producer of a unique human retrovirus. Of very great interest are Dr. Gallo's laboratory findings of the uniqueness of this virus, and the fact that patients with MF-SS have antibodies against viral polypeptides. CTCL may represent the most unique and best opportunity yet to study the role of retroviruses in the etiology and seroepidemiology of a human malignancy. Thus, these patients have to be given very high priority. In addition, the cytogenetic evolution of this lymphoma is very interesting and in contrast to other malignant diseases and we are studying this in collaboration with Dr. Wheng Peng of the Medicine Branch.

Our first clinical trial in CTCL involved the study of combined modality treatment with electron beam radiation and combination chemotherapy (NCI-VA Protocol 76-12) in collaboration with WVMC Hematology (Dr. Schechter), WVMC Dermatology (Dr. Fischman) and Howard University Radiotherapy (Dr. Goldson and Kumar), and the Radiation Oncology Branch. Preliminary results of the trial have been published. We have found excellent results in stage I disease with 71% complete response rate and no relapses to date. In comparison with the Stanford data the NCI trial has slightly better survival and dramatically better (71% vs. 39%) disease free survival. In more advanced stages we have found a much lower complete response rate (28%) and nearly all of these patients have relapsed but the overall survival (median of 3 years) has been excellent and either equivalent or better than the results at other centers. This finding is advanced stage disease again mimics the nodular B cell lymphomas. The toxicity of this therapy has been

primarily cutaneous. We concluded from this first study that combined modality therapy is highly effective in producing objective tumor remissions which palliates but is so far not effective in advanced stage disease in curing patients.

These first studies have led into the current trial a randomized comparison of conservative vs. aggressive combined modality therapy (NCI-VA Protocol 79-2) in collaboration with the Radiation Oncology Branch and the WVAMC Sections of Dermatology and Hematology. Patients are initially staged and randomized to receive combined modality therapy with electron beam irradiation and much more intense combination chemotherapy with cyclophosphamide, adriamycin, VP-16, and vincristine (CAPO) with curative intent vs conservative treatment with the standard approach of sequential topical therapies including Topical Nitrogen Mustard, Photochemotherapy with methoxsalen and ultraviolet light (PUVA), and spot electron beam therapy. So far 23 patients have been randomized on this trial which we plan to continue for all of the reasons listed above.

In addition to the primary trials in CTCL we are also conducting phase II trials of antithymocytglobulin, and plan to begin trials with monoclonal anti-T cell antibodies. These diseases may be ideal first candidates for such immunotherapy trials using these new monoclonal reagents. Other studies which include phase II trials of VP-16-213, and high dose methotrexate with leucovorin rescue are also ongoing.

Our trial in prostatic carcinoma is a unique test of the efficacy of chemotherapy prior to hormonal manipulation (in standard treatment hormonal manipulation is given first). This allows testing for the presence or absence of cross resistance of hormonal and combination chemotherapy. In NCI-VA Protocol 77-8 in collaboration with Diagnostic Radiology, CC, NIH, the Medicine and Surgery Branches, and the Urology Service of the WVAMC we have been exploring the role of combination chemotherapy with cyclophosphamide, adriamycin, and cis-Platinum (CAP) followed by hormonal therapy for patients with previously untreated disseminated extrapelvic carcinoma of the prostate. In prior work at the NCI-VA Branch we have shown that by the use of extensive serial staging procedures we can objectively document tumor response. We have added to this pre and post treatment percutaneous prostatic biopsies to determine sex steroid receptor proteins which we are attempting to correlate with tumor response. So far 14 patients have entered the trial and 4 patients have objective partial tumor responses. In addition, patients progressing on chemotherapy have responded to orchiectomy. We have thus concluded that: 1) initial combination chemotherapy does not result in a very high response rate to combination chemotherapy and; 2) that there is not cross resistance to hormone therapy. Following completion of this trial we plan a randomized trial of hormonal therapy with initial combination chemotherapy vs. chemotherapy at the time of progression in prostate cancer.

In addition, we are performing a phase II trial of combination chemotherapy with cyclophosphamide, adriamycin, and cis-platinum for hormone-resistant prostatic carcinoma (NCI-VA Protocol 77-8). Thus, we will be able to compare the program's relative efficacy in patients resistant and sensitive to hormonal manipulation.

A major primary treatment trial involved treatment of multiple myeloma with alternating combination chemotherapy (NCI-VA Protocol 76-7) in collaboration with the Mematology Vection, WVAMC (Dr. Schechter). The purpose of this trial was to treat with alternating combinations of non-cross resistant agents and to test whether this scheme would increase the complete response rate (rare in multiple myeloma), prolong the time to disease progression, and increase survival compared to historical controls. After 27 patients we have concluded that this regimen does not increase the complete remission rate, or give increased reduction in M protein levels which reach a plateau at 6-9 months into therapy as in other studies. The survival is equivalent to the best reported results elsewhere.

We have conducted a phase I-II trial of low dose total body radiotherapy (TBI) in patients with multiple myeloma and waldenstrom's macroglobulinemia failing primary combination chemotherapy, in collaboration with the Radiation Oncology Branch. This trial is just beginning.

We are in the process of designing a new trial in multiple myeloma in collaboration with the Radiation Oncology and Pediatric Oncology Branches to test the effect of interferon on multiple myeloma, and the effect of TBI given during remission. This protocol is under development.

We are conducting a phase II trial of adrimaycin and streptozotocin

(NCI-VA Protocol 76-11) in hepatocellular carcinoma. Fifteen patients have been treated with two partial responses. This is not significantly different than with adrima adriamycin alone. We plan to continue phase II trials of interesting agents, selecting therapy based on in vitro tumor colony forming assays because of the ease of tumor cell availability by peritoneoscopy and multiple liver biopsies in this disease.

In collaboration with the Clinical Pharmacology Branch and the Medicine Branch we are conducting a series of Phase I trials. These include dicloro-methotrexate (DCM, NCI-VA Protocol 78-1. Because of significant hepatic metabolism DCM would be more suitable than methotrexate for administration to patients with impaired renal function. 17 patients have been treated, a dose limiting toxicity range of 1200 mg/M² IV q-week had been established, and objective responses have been seen in adenocarcinomas of unknown primary site. Thus, this agent is now ready for phase II trials.

We have completed a phase I trial of Pentamethylmelamine (PMM, NCI-VA Protocol 78-5) the water-soluble analog of hexamethylmelamine suitable for intravenous administration which should have considerable clinical interest. A dose limiting toxicity of nausea and vomiting was reached at 1500 mg/M² (with a recommended Phase II trial dose of 1000 mg/M² per week) as well as minimal lethargy and somnolence, but consistent myelosuppression was not observed. In addition, the IV route of administration because of the lack of myelosuppression PMM will become a very interesting agent if anti-tumor activity can be defined at tolerable doses.

The current phase I trial is of aziridinylbenzoquinone (AZQ) NCI-VA Protocol 80-2) in collaboration with the Clinical Pharmacology, Medicine, and Pediatric Oncology Branches. This drug is of interest because of its good lipid solubility, good water solubility and low ionization which may allow it to penetrate the central nervous system. It is believed to function as an alkylating agent.

In collaboration with the Biometry Branch (D. Makach) we have been analyzing the influence of sites of metastases on prognosis in patients with SCCL. The results from this analysis demonstrate that: initial performance status is a dominant prognostic fact; the number of sites of metastatic disease correlates with performance status but patients with a single site of distant metastatic disease have similar survivals to limited state patients; and that liver and central nervous system involvement independently effect survival when other factors are statistically accounted for. These findings have been corroborated by several other institutions, and together, they will form the basis for new staging system, design of future clinical trials, and provide a better understanding of the natural history of SCCL. In addition, they demonstrate the benefit of statistical collaboration and the extensive pre and post treatment staging studies performed by the NCI-VA group.

Several studies continue to evaluate the utility of staging procedures and tumor markers in the natural history and management of malignant diseases. The unit has pioneered the use of fiberoptic bronchoscopy (FO) in the initial treatment of SCCL, and has demonstrated the importance of a histological and visual complete response in material obtained by FOB in the survival a SCCL

patients. In non-SCCL we have recently published our FOB results and have shown that 40% of early tumor progressions in this disease were detected by FOB alone. This allows the early discontinuation of ineffective drug therapy, and potentially will allow access to tumor material for in vitro drug sensitivity testing.

Peritoneoscopy with multiple liver biopsies is being investigated as a staging and restaging procedure in patients with SCCL and cutaneous T-cell lymphomas. In SCCL, pretherapy staging peritoneoscopy has been performed in 60 patients with negative percutaneous liver biopsies and we have found many additional patients with unsuspected liver involvement. We are correlating these results with survival and also studying the tumor material obtained in the human tumor colony forming assay and by flow cytometric studies. In CTCL peritoneoscopy appears to be the procedure of choice for staging the liver.

We have been using staging studies to follow the development of metastases in sanctuary sites (especially the central nervous system) in SCCL and CTCL. At the present time CT scans are the best way to detect lesions in asymptomatic patients while radionuclide scans are of no value. Prior work by our unit has shown the high rate (up to 80%) of CNS metastases developing in long term survivors, unless prophylactic therapy is given. In addition, we have reported on the development of leptomeningeal and spinal epidural metastases in these patients and the subsequent response to intrathecal therapy. We are collaborating with the Clinical Pharmacology and Medicine Branches in testing new types of intrathecal chemotherapy.

In cutaneous T-cell lymphomas we have found that osteoporosis develops in several of these patients and in collaboration with Drs. Schechter and Horton that these tumors may be secreting osteoclast activating factor ("OAF"). We have also found that pulmonary and leptomeningeal involvement is common in these diseases. A clinical review of the natural history and treatment of CTCL has been published by Drs. Carny and Bunn.

In collaboration with the Diagnostic Radiology Division (Dr. Dunnick) of the Clinical Center and Dr Bates of the Nuclear Medicine Service (WVAMC) we have been evaluating computerized (CT) and radionuclide scans of abdomen, pelvis, and brain in various malignancies. We have found unsuspected abdominal disease in asymptomatic SCCL patients with tumor in liver, adrenals and retroperitoneal masses, and a preliminary report has been published. We found, CT scans are complimentary to radionuclide scans in evaluation of hepatocellular cancer patients, and we are studying other GI malignancies with this technique where objective tumor responses are traditionally difficult to document by other methods.

With the Diagnostic Radiology Division/CC we have been studying the role of lymphangiography (LAG) in prostate cancer and mycosis fungoides-Sezary Syndrome patients. The LAG's have yielded evaluable evidence of nodal metastases in 60% of the prostate patients, and have shown positive nodes in 70% of mycosis fungoides patients if peripheral nodes are palpable. We are continuing to use these markers as objective indicators of response to therapy.

Tumor markers which we are following in serial serum samples in our patients in collaboration with Dr. Ruddon, Frederick CRC, Dr. McIntire of the LID/DCBC, and Dr. Zweig, Clinical Chemistry, CC include: a lung cancer associated antigen; calcitonin, other polypeptide hormones; prostatic acid phosphatase; by RIA;; and of great interest the BB isozyme of creatine kinase. This latter enzyme appears to be a marker of both prostate cancer, and SCCL.

With the use of intense combination chemotherapy and radiotherapy treatment related complications increase. Overall, in SCCL in over 100 patients treated with CMC-VAP we have found an early (two month) treated related mortality rate of 6%. With the use of the multiple (3-6) induction cycle regimen of CAPO this rose to 18% (clearly unacceptable). Thus, we remain interested in studying the various acute complications of treatment and providing supportive care during this period. While we removed the laminar air flow rooms in 1976 because of their great expense, and the lack of any demonstrated value in a prospectively randomized trial we conducted we are still interested in the study of infectious complications. Dr Fossieck has reported recently on 45 cases of Staphylococcus aureus bacteremia occurring in patients with solid tumors and cutaneous T-cell Lymphomas. In collaboration with the Infectious Disease section WVAMC he has investigated the use of oral antimicrobial therapy for staphylococcal sepsis compared to intravenous therapy and found that oral therapy was as effective. This oral therapy can avoid the morbidity, limitation of activity, and extended hospitalization associated with prolonged intravenous drug administration.

Counterimmunoelectrophoresis for the detection of staphylococcal teichoic acid antibodies was tested in 216 cancer patients but was found not to be useful in establishing or excluding the diagnosis of serious S. aureus infection in this group of patients.

Because of our prospective randomized combined modality trial in cutaneous T-cell lymphomas Dr. Fossieck has investigated a major problem, the septicemic complications of this disease, and its therapies. Gram positive coccal infection was common, but was associated with a significant number of gram negative bacillary superinfections which thus require multiple drug therapy.

While fatal anorectal infections are known to occur in 10-30% of patients treated for acute leukemia their prevalence in solid tumors treated with intense therapy is not well recognized. We have found the incidence to be 6% in solid tumor patients with a high mortality and major risk factors for this were a prior history of anorectal pathology and of course severe granulocytopenia. Thus, we are carefully monitoring in a prospective fashion our patients for this complication.

A prospective study of hepatitis virus transmission in our unit in collaboration with the GI-Hepatology section WVAMC revealed no evidence for such transmission in patients or personnel, and important finding.

Diffuse interstitial pulmonary disease associated with combination chemotherapy with or without radiotherapy remains a serious problem, occurring in 10-20% of patients. Biopsies of such patients has not revealed treatable

infectious etiologies. Thus, we are starting a prospective collaboration with the Radiation Oncology Branch, and Clinical Pharmacology Branches all of which have expertise in lung toxicity of the various therapies to identify and correct if possible this pulmonary toxicity which we feel is now the major limiting toxicity in the treatment of patients with lung cancer with intense combined modality therapy. Dr. Cohen's laboratory work with changes in red cell deformability appear to relate directly to this as patients with low red cell deformability have a very high incidence of lung toxicity.

In long term survivors the development of hemotologic abnormalities including preleukemia, and overt leukemia are real concerns which we have already seen and reported on. We are prospectively monitoring our patients in collaboration with the Cytogenetics section of the MB, as well as beginning investigations in the laboratory to identify early leukemogenic steps in patients treated with and without radiotherapy.

We have begun substantial efforts in the rehabilitation and nutritional support of cancer patients. This includes enteral nutrition during intensive induction combination chemotherapy and combined modality therapy, and evaluation in collaboration with the Dental Service WVAMC of mucous membrane prophylaxis.

The most important supportive care finding was made in the long term survivors when one of our research nurses (Ms. A.J. Early) discovered that these patients had stopped smoking. This led to a prospective and retrospective evaluation of the effects of cessation of smoking on the survival of SCCL patients. In collaboration with the Biometry Branch we have found in 112 newly diagnosed SCCL patients that the best survival occurred in patients who had stopped smoking prior to diagnosis, the next best in patients who stopped at diagnosis, and the worst in patients who continued to smoke. We plan to continue the prospective study of this simple but apparently very important factor in the survival of SCCL patients which appears not to have a trivial explanation (eg. poorer risk patients or more lung infections in the smoking groups).

The NCI-VA Branch is particularly fortunate to have two pathologists on its senior staff, Dr. Mary Matthews, and Dr. Adi Gazdar. While Dr. Gazdar has his main responsibility the administration of the laboratory research portion of the branch, Dr. Matthews is in charge of the pathology work, clinical lab of the unit, and responsible for the clinical pathologic correlations in patients with malignant disease. It should be noted that Dr. Matthews is a pathologist with a world wide reputation in lung cancer and one of the central figures in the new WHO classification of lung cancer. In addition to review of all pathologic materials on NCI-VA Protocol patients her reference and consultant pathology roles include: directorship of the NCI registry of long term survivors of SCCL, VA surgical Oncology Lung Group, Eastern Cooperative Oncology and Radiation Therapy Oncology Cooperative Groups for small cell carcinoma; NCI-Lung Cancer Study Group; VA Surgical Oncology B Group; GI Tumor Oncology Group for Pancreatic protocols; NCI SCCL hyperalimentation protocol.

IN work done specifically on NCI-VA Protocol patients Dr. Matthews and Dr. Gazdar have studied the clinical pathologic correlations of therapy response natural history and subtype of SCCL. They have found no differences between all subtypes except for the subtype with mixtures of small cell carcinoma and large cell carcinoma (WPL# 22/40). This group had an objective response rate of 56% compared to that of other SCCL subtypes of 90% and a significantly inferior median survival. However, long term disease free survivors of this subtype exist.

Dr. Matthews has studied intra and interobserver variability in the histopathologic classification of lung cancer, and subtypes of SCCL in collaboration with the Finsen Institute, Yale, Albert Einstein, Univ. of Rochester, Johns Hopkins Cancer Center, Mt. Sinai Hospital New York and the WVAMC.

A large number of diagnostic lung cancer slides were coded and a panel of eight pathologists independently reviewed the material for diagnosis. The results of this important study showed: that in general there was good classification of SCCL from non-small cell lung cancer. However, in up to 25% of cases there was not unanimity in this basic distinction which has tremendous therapeutic implications. The types of lung cancer causing problems including poorly differentiated adenocarcinoma, poorly differentiated epidermoid cancer, and large cell carcinoma. This study stresses the importance of a pathology panel for review, the necessity for standard criteria, and the need to develop immunochemical and biochemical markers of the different types of lung cancer. We are developing such markers using monoclonal antibodies and in studying the expression of APUD properties in our laboratory work. In contrast, the subtyping of SCCL was highly variable with unanimity in only 40-60% of cases. For this reason, at present the NCI-VA group strongly recommends not to base treatment decisions on the subtype of SCCL with the possible exception of the small group of patients (6% of all protocol entries for SCCL) with mixtures of small cells and large cells.

We have noted the development of different lung cancer histologic types after treatment with chemotherapy and or chemotherapy and radiotherapy. This was seen in living patients, at autopsy, and also in laboratory work in cells cultured in vitro and in vivo in nude mouse xenografts. This transformation has led Dr. Gazdar and Dr. Matthews to suggest that all histologic types of lung cancer may be entodermal rather than some being neuroectodermal in all origin. Thus, all lung cancer may have a common stem cell, a situation analogous to the spectrum of diseases seen in the B cell lymphomas. They also suggest that such a transformation may be a mechanism of resistance. Thus, we are interested in modifying the biologic response of these tumors to try to prevent the shift in state of differentiation from small cell (therapeutically responsive to large cell (therapeutically unresponsive).

The NCI Small Cell Cancer Registry of Long Term Survivors has provided independent documentation of the possibility of cure of this disease using several different treatment modalities including surgery, radiotherapy, and chemotherapy often in combination. By review of pathologic materials Dr. Matthews has found that all subtypes of SCCL are represented in the cured patients, another indication not to base treatment decisions on histologic subtype.

In examination of the VA Surgical Oncology Adjuvant study materials Dr. Matthews in collaboration with Drs. Higgins, Shields, and Mr. Keehan has found that histologic type of non-small cell lung cancer is important. When lymph nodes are involved, adenocarcinomas and large cell carcinomas have inferior survival compared to squamous cell cancer, and large cell carcinomas may have the worse survival.

Histologic criteria for skin and lymph nodes in cutaneous T-cell lymphomas are being studied in collaboration with WVAMC Dermatology (Dr. Fischmann), Hematology (Dr. Schechter) and Laboratory Service

(Drs. Guccion, Patterson, and Ortega). A coded slide study has been performed that has helped to define criteria.

Another area of interest is the histologic study of lymphomatoid granulomatosis, a disease often terminating in malignant lymphomas. 49 cases of this relatively rare disease have been collected for pathologic review and for clinico-pathologic correlations. This preliminary study should lay the foundation for a multidisciplinary interest in this disease.

The NCI-VA nurses have been active in local and national organizations. The research nurses play an important role in the conduct of the clinical trials and are authors on many of the papers. Several original observations (eg. effect of smoking on survival in SCCL) have been made by these nurses. A group of NCI-VA nurses attended the national meeting of Oncology Nurses in San Diego and presented several papers. They have also published several papers on their work on the NCI-VA protocol patients in nursing journals.

Dr. Alvarez, Dr. Oie, Dr. Gazdar, Dr. Minna

We are continuing our work on the genetic analysis of malignancy of human lung cancer using hybrid cells and other gene transfer techniques. Somatic cell hybridization is an important technique for genetic analysis of malignancy. Hybrid cells formed between malignant human cells and non-malignant rodent cells can be tested for tumorigenicity in nude mice and for replication in low concentrations of serum and the ability to form colonies in semi-solid medium. The retention or loss of specific human chromosomes can be correlated with the expression or suppression of the malignant phenotype. It turns out that human lung cancer cells particularly SCCL are difficult to fuse to normal rodent cells to generate replicating hybrid cell lines. We are investigating the basis for this hybrid sterility which in itself may have important biologic consequences (for example, previous work of ours has shown in some lung cancer x mouse crosses that malignancy is suppressed and it is possible that the rodent genes are turning off the lung cancer cells ability to divide in vitro). However, we have isolated some hybrids and we are beginning characterization of these.

Recent advances in gene transfer technology have allowed investigators to transfer isolated DNA using coprecipitation with calcium phosphate and in some cases DNA from malignant cells have been used to transfect and transform non-malignant cells into malignant variants. We are beginning such experiments with DNA from lung cancer cells. If such DNA transfer experiments work they will allow subsequent isolation of the genes coding for malignancy by recombinant DNA techniques.

We are also planning to transfer genes related to APUD differentiation (for example, ACTH, Insulin) that have been cloned in plasmids into the SCCL and assay their expression.

Dr. Alvarez, Dr. Oie, Dr. Radice, Dr. Gazdar, Dr. Cutitta, Dr. Minna

In addition to the use of hybrids for identifying chromosomes which code for malignancy, our laboratory is attempting to use this technology to identify chromosomes which code for the production of polypeptide hormones such as ACTH, endorphin, calcitonin, and AVP, as well as other APUD markers (L-dopa decarboxylase, and the BB fraction of creatine phosphokinase). All of these markers are expressed by our SCCL tissue culture lines. Other markers which we can map are the antigens marked by monoclonal antibodies that are present on human lung cancer cells and either related to the malignant phenotype or related to APUD differentiation. We wish to see if these markers are dominantly or recessively expressed and whether they are located on a few or many different human chromosomes.

Other potential use of hybrid cells made with human lung cancer include their use as immunogens in the production of monoclonal antibodies after human chromosome segregation has reduced the human gene content, and the possibility that hybrid cell technology could be used to rescue human tumor cell stem cells for drug sensitivity testing.

Dr. Minna, Dr. Oie, Dr. Alvarez, Dr. Radice, Dr. Cutitta

Prior work from our laboratory has shown that differentiated functions from normal cells can be rescued in replicating hybrid cells by using a tissue culture adapted line that is differentiating along the same pathway as the normal tissue of interest. While hybridomas producing specific antibody are the best example of this, we have shown that neuroblastoma cells fused to the normal nervous system can also rescue new functions related to neural differentiation. We are trying to extend this by fusing APUD derived tumor cell lines from man and mouse (including SCCL, pheochromocytoma, insulinoma, and pituitary tumors) to normal cells of the APUD system and then scoring the resulting hybrids using radioimmunoassays for the production of new polypeptide hormones. This approach should have far reaching consequences in medicine and biology for generating novel cell lines and protein products. At least some of the products we will be testing for include peptide hormones that can regulate the growth of human lung cancer cells (Biologic Modifiers).

Dr. Oie, Dr. Gazdar, Mr. Simms

In collaboration with Dr. Chick (Harvard Med. School), Dr. Weir (Virginia Commonwealth Univ), and Dr. Recant's group (Diabetes Res. WVAMC), we have established, cloned and characterized a rat islet cell tumor line. We have shown these cells express the APUD program of differentiation including formaldehyde induced Fluorescence (FIF), and DOPA decarboxylase activity, and that they produce insulin and somatostatin. Analysis of the clones show that variants secreting no hormones, both hormones, or only one hormone have been isolated. By subcloning we have shown which steps in the program of differentiation are reversible and which appear to be relatively stable.

We have also found glucagon secretion in some lines; we have studied the effect of secretagogues on hormone secretion and find them to be similar to normal islet cells but in some instances novel effects are found. These clones provide important tools for the study of mechanisms controlling hormone secretion by different types of islet cells.

The cells also have specific receptors for insulin, somatostatin, and glucagon. The presence of specific receptors for all three hormones in parent and subclones alike support the likelihood that hormone regulation among the islet cells is probably mediated by all three hormones. These clones will also be genetically studied using hybrid cells as discussed previously.

Dr. Oie, Dr. Gazdar, Mr. Simms

In collaboration with Dr. Reid (Albert Einstein U.) we have established a human pheochromocytoma line in culture and began characterization. These cells also express APUD properties and have high levels of enzymes involved in catecholamine metabolism. These cells will also be used for cell hybridization experiments and will be compared to the SCCL cell lines to see how their APUD programs differ.

Dr. Bunn, Mr. Schlamm

We have investigated the DNA content and cell kinetics of tumor cells taken directly from patients with lung cancer. We have shown that the majority of SCCL and nearly all non-SCCL were aneuploid, and in all cases this aneuploidy was hyperdiploid in nature. We have compared the DNA content of the original tumor cells to those of the cells grown in culture, soft agar, and in nude mice and in all cases they were similar proving that the DNA content is a reliable signature of the tumor cell lines. In some cases multiple stem lines were found and these stem lines could be separated by cloning. It appears that non-SCCL tumor cells have higher DNA contents than SCCL, a point which may be of diagnostic importance. In comparing the results of flow cytometry with standard pathology is found an excellent correlation and that the discrepancies resulted from diploid tumors (detected by pathology) or the presence of very few tumor cells (detected by FMF) and thus the two methods are complementary.

We have also studied the cell cycle distributions of lung tumors from patients and tissue culture systems, and nude mouse heterotransplants. The doubling times of the SCCL tumor cells are slightly faster than the non-SCCL tumors, but the cell cycle distribution parameters are not yet significantly different. We have recently acquired a new multiparameter cell sorter and we plan to analyze our lung cancer cells from these systems with dual parameters including using monoclonal antibodies that are specific for human lung cancer cells. Using this instrument we plan to study the effect on cell kinetics of drugs, and biologic response modifiers on tumor cells taken directly from patients, or after isolation in semi-solid medium or the defined serum free, hormone supplemented selective medium we have developed. We plan to correlate the DNA content analysis with cytogenetic analysis by Dr. When-Peng.

Dr. Bunn, Mr. Schlamm, Dr. Gazdar

We have shown by cytogenetic and FMF studies that 90% of patients with cutaneous T-cell lymphomas have aneuploidy. About 15% of patients have marked hyperdiploidy and these patients have a worse prognosis than the other patients. We are studying the histologic conversion of malignant T-cell lymphomas from small to large cell size variants using FMF.

Dr. Bunn, Dr. Cutitta, Dr. Minna

In collaboration with Drs. Mann, Haynes, and Fauci of the NIH and Dr. Royston (UCSD) we have been studying the binding of monoclonal antibodies to cutaneous T-cell lymphomas in preparation for a therapeutic trial using monoclonal antibodies and to dissect the T cell subset found in CTCL. We have found that T-cells from our patients do not express the 3A1 monoclonal T-cell antibody determinant present on the majority of normal T-cells., but they do express the 4F2 antibody determinant which is associated with rapid proliferation of human cells. Dr. Fauci's group is correlating the antigen expression with various T-cell functions. We plan to continue this work because of the unique nature of the monoclonal antibody reagents, and the patient CTCL material.

Dr. Bunn, Mr. Schlamm

We have found, using FMC and cytogenetides, that 50% of multiple myeloma patients at diagnosis have aneuploidy and these patients have a worse prognosis compared to patients with diploid tumors initially. At relapse using FMF we can detect clone formation and this is associated with a very bad prognosis. Dual parameter studies in collaboration with Dr. Alabaster, NIH and George Washington University have allowed separation of normal from malignant plasma cells and we plan to continue these studies with a particular emphasis on the clinical management of patients.

Dr. Bunn, Mr. Schlamm

In collaboration with Dr. Levine (WVAMC Hematology) we have been studying megakaryopoiesis using FMF. We have shown that FMF is perhaps the best way to follow the ploidy differences during megakaryopoiesis, and we compared this in several species. We plan to extend this by using dual parameter sorting involving DNA content and platelet specific antibodies to study megakaryopoiesis in patients.

Dr. Cohen

We have been studying tumor blood flow in experimental tumor models. Capillary blood flow is primarily determined by red cell deformity and so we are focusing on this determination. We have found that in animal tumor models and in patients with SCCL that red blood cell deformability decreases with increased tumor burden. We have corrected this in part by the use of inosine, adenosine glucose, sodium pyruvate or ascorbic acid along with chemotherapy in the animal models. These additions resulted in significant therapeutic benefit.

We have tried to relate the toxicity of chemotherapy to red cell deformability because this may increase the toxicity on normal tissues particularly the lung. We have found that the toxicity of intensive induction chemotherapy in SCCL was significantly related to decreased red cell deformability with the least deformed red blood cells coming from patients who would then develop the most toxicity. Thus, we are planning to use this as a predictive test in patients.

Dr. Cohen, Dr. Matthews

We have been studying experimental animal models of diffuse interstitial pulmonary disease associated with antineoplastic therapy. Such interstitial pulmonary disease is becoming a major complication of therapy. We have developed a murine model and found that Mitomycin-C, BCNU and cyclophosphamide cause pulmonary toxicity while adriamycin did not. We have found the damage is related to tumor bulk, and scheduling of therapy. The main approach is to test drugs that will improve red cell deformability, in addition to standard cytotoxic chemotherapy in the animal models. We are planning to continue to study this model system in an attempt to apply these results in human patients.

1.9 Summary of Laboratory Investigations

In the laboratory, we are studying fundamental questions of tumor cell biology in an attempt to develop new methods to treat human cancer. Areas of investigation include: cell biology studies in vitro and as nude mouse hetero-transplants in vivo by establishing continuous cell lines; clonogenic assays of human tumor cells; identification of hormone and growth factor requirements of the human tumor cells; study of the expression of differentiated functions by human tumor cells and the regulation of this differentiation; development of hybridomas producing monoclonal antibodies with specificity for human tumor cells or the differentiation antigens they express; study of human tumor cell kinetics in vitro and in vivo; experimental chemotherapy in xenografts and other animal models; study of blood flow of tumors and experimental models of pulmonary toxicity from therapy. The major tumors we are studying in the lab are derived from lung, and adult cutaneous T-cell lymphomas.

Dr. Gazdar, Dr. Carney, Mr. Russell, and Dr. Minna

We continue to spend considerable effort in establishing and characterizing continuous cultures of lung cancers, both small cell (SCCL) and non-small cell carcinoma of the lung. After many attempts to generate continuous cell culture line of SCCL only 2 of 21 specimens plated in fetal calf serum (FCS) supplemented medium developed into lines. In contrast, when we added media conditioned by other small cell lines 8 of 13 specimens developed into lines. This conditioned medium (CM) also increased the colony forming efficiency (CFE) of primary tumor specimens and early unestablished cultures. From these simple experiments we conclude that it is likely that SCCL can produce the growth factors required for its own continuous replication in vitro but that these are not always expressed in tumors taken directly from patients. This is in direct contrast to the ready growth of melanomas and soft tissue sarcomas in culture and has potential therapeutic applications; namely, can these growth factors requirements be manipulated in the patient? In contrast to SCCL, three of five non-SCCL tumors were readily established as continuous cell lines in regular FCS supplemented medium. Thus it is possible that non-SCCL tumors provide their own growth factor requirements in the patients and this may be related to their relative unresponsiveness to cytotoxic chemotherapy in patients.

Dr. Gazdar, Dr. Carney, and Mr. Simms

A large number of lung cancer specimens have been injected directly into athymic nude mice and 37% produced progressively growing tumor xenografts. However, when we try to culture these tumors, their replication in culture for periods of 3-24 months remains strictly dependent on mouse cells feeding them. Thus, in both specimens put directly into culture from patients, or those introduced into culture from nude mouse heterotransplants, there were stringent requirements for growth factors to allow replication. By injecting tumor cells intracerebrally into nude mice we have found that 1000 fold less human tumor cells can form tumors. Thus, this is an important way to increase the take rate, and to transfer individual colonies from the human tumor colony forming assay into the nude mouse to develop cell lines.

Dr. Gazdar, Dr. Carney, Dr. Alvarez, Dr. Oie, Dr. Radice, Dr. Bunn and Dr. Minna

We have found that small cell lung cancer cells lines continue to express a program of Amine precursor uptake and decarboxylation (APUD) differentiation in vitro and as nude mouse heterotransplants while non-small cell carcinoma of the lung do not express this program. The APUD markers include high specific activities of the enzyme L-dopa decarboxylase, high specific activity of the BB isozyme of creatine kinase, SCCL histology, formaldehyde induced fluorescence (FIF), neurosecretory granules by electron microscopy, and the secretion of polypeptide hormones. In culture they have a long population doubling time of 70-150 hours, with a colony forming efficiency of 1%. These findings indicate that the number of stem cells in the population is low, and that the number of stem cells in the population is low, and that cell death is high. In contrast non-SCCL cultures had faster population doubling times, and apparently less cell death, with 10 fold higher CFE. Thus, there appear to be biologic reasons to account for the differences in sensitivity to therapy of the SCCL vs. the non-SCCL histologic types. These studies have been done in collaboration with Dr. Baylin (John Hopkins Cancer Center), Dr. Guccione (WVAMC); Dr. Zweig (Clinical Chemistry CC, NIH).

Dr. Radice, Dr. Gazdar, Dr. Alvarez, Dr. Carney, Dr. Oie, and Dr. Minna

We have found that SCCL tissue lines appear to be heterogeneous with respect to polypeptide hormone production. In collaboration with Dr. Dermody of the FCRC, and Dr. Becker of the Endocrinology Section (WVAMC) we have studied the primary lines, and many clonal sublines and assayed them for production of ACTH, calcitonin, arginine vasopressin (AVP) as well as other APUD markers. While 75% of the lines produce at least one polypeptide hormone, we have found considerable heterogeneity in the subclones of different lines. All possible combinations of the ACTH, calcitonin, and AVP were found. In fact, clones from lines not producing these polypeptid hormones were found in some cases to turn on their production. We thus conclude that despite the sensitivity of radioimmunoassays in detecting these polypeptide hormones, their expression may be so heterogeneous that they will not be good markers to follow in patients. We are planning to study the regulation of the expression of the markers and the precursor forms.

Dr. Gazdar, Dr. Carney, and Mr. Simms

During the course of cloning studies on the SCCL tissue culture lines and the transplantations of the xenografts we discovered that SCCL can transform to a large cell variant. This appears also to occur in patients (See clinical section) and is associated with resistance to chemotherapy. Histologic examination revealed these transformants to have the appearance of large cell carcinoma, their cloning efficiency was ten fold increased, and their population doubling times more rapid than the original SCCL cultures. In addition, they failed to express the markers of the APUD program including L-Dopa decarboxylase, FIF, and neurosecretory granules. Thus, their marked change in biologic behavior may account for their clinical course in patients. These findings also suggest that all lung cancer types may come from a single type of progenitor cell because in some instances squamous differentiation is seen as well as adenocarcinoma type differentiation.

Dr. Abrams, and Dr. Minna

We have studied the replication of our SCCL cell lines in serum free defined medium supplemented with various hormones. By a series of additional experiments we have found the combination of selenium hydrocortisone, insulin, and transferrin, and 17 B-esbradiol (SHITE) to medium RPMI-1640 allows optimal replication of these cells when transferred directly from serum containing medium. We have maintained SCCL lines in this medium for almost one year, and they have been found to retain their histology, tumorigenicity and continue to express their APUD program and can clone in this medium. The formula appears to be a general one for all SCCL lines tested. The identification of this growth formula will allow study of hormone production and regulation of these cell lines, the identification of growth regulatory substances, and therapeutic agents. In fact, we have found that certain hormones, when added to the serum free SHITE supplemented medium inhibit the growth of SCCL and thus are biologic response modifiers. These include epidermal growth factor, LH releasing factor, and the liver cell growth tripeptide Gly-Hys-Lys. This formula should allow for the isolation of the normal tissue precursors of SCCL and the study of their biology and potential conversion to tumor cells under the influence of carcinogens.

Dr. Gazdar, Dr. Carney, Mr. Russell and Dr. Bunn

We have continued to try to establish continuous cell lines from malignant cutaneous T-cell lymphoma tumors. These cell lines are in great demand for the study of T-cell biology and because one of them has been found by Dr. Gallo's laboratory to produce a unique retrovirus. We have established a method using first the determination of mitogen responses by tritiated thymidine incorporation and flow cytometry, and then selecting the optimal mitogen from a panel of five. These T-cell lines grow free of the T-cell growth factor (TCGF) and continue to express T-cell but not B-cell properties. They may themselves produce TCGF and other lymphokines and thus they may be an extremely important source of TCGF for other lymphokines and thus they may be an extremely important source of TCGF for other studies. In collaboration with Dr. Gallo's laboratory (Dr. Posiez, Dr. Russiutti, Dr. Posner and others) this cell line was studied for viral production. Dr. Posiez discovered the presence of a unique retrovirus isolate HTCLV strain CR from patients CR's cell line (NCI-H-102). They have shown that it is biochemically and immunologically distinct from other retroviruses, possesses a reverse transcriptase, endogenous RNA, and has characteristic retrovirus morphology. They have carried on an extensive characterization of the virus. Dr. Posner has found that patient CR and other MF CTCL patients have antibodies in their serum to this virus particle. Thus, this provides an excellent opportunity for seroepidemiology of this virus, and should lead to finding out whether it is related to the T-cell lymphomas.

Dr. Carney, Dr. Gazdar, Dr. Bunn, and Dr. Minna

We have established a human colony forming assay in soft agar to detect human tumor colony forming units (TCFU's) in samples taken directly from patients. This system is based on the selective growth of tumor cells in semi-solid medium (non-anchorage dependence). Evidence from other groups

suggests that this may be useful in predicting drug sensitivity in vitro and thus is of great therapeutic importance. In addition, it can be used to detect biologic modification by hormones and other agents promoting differentiation.

We first optimized the assay because the original assay of Hamburge and Salmon either failed to grow SCCL or had a very low efficiency. We have increased the CFE by 10 to 100 fold. Next we determined whether the cells growing in agar were tumor cells or not. We proved this by cytologic examination, the presence of aneuploid DNA content, and their ability to grow in nude mice tumors. Next we tested specimens from 91 patients with SCCL and correlated the TFCU's with TCFU's with histo-cytopathologic examination of the specimens. We found excellent correlation, and in some cases detected tumor cells not found by standard histology. We have calculated the CFE per plated SCCL tumor cell and this ranged from 0.05 to 1.5%, very similar to that observed with the established SCCL lines. Thus, it is likely that the vast majority of tumor cells seen in clinical specimens are not stem cells. However, the possibility still exists that we have not yet optimized growth conditions and we are continuing to study this. One approach has been to add conditioned medium from the established SCCL cell lines grown in tissue culture. This has doubled the CFE and has dramatically improved the growth of the TCFU's.

We have investigated the "Stem Cell" nature of the TCFU by transferring individual colonies to microwell culture. However, in over 135 examples we have not seen continued growth. However, when we transplant these to nude mouse brain intracranial, a tumor forms. Thus, we feel these may indeed be stem cells but they have such stringent growth requirements (which cannot be replaced entirely by conditioned medium) that their replication is dependent on exogenous ingredients. We are trying to use the TCFU transfer to microwells as an assay to identify these growth factors and requirements.

We are beginning drug sensitivity testing in vitro using the TCFU assay to see if it will predict for drug sensitivity. We plan first to correlate the response of untreated patients with their in vitro responses and to search for drug sensitivity with the assay in released ACCL patients. If these tests appear predictive we plan ultimately to use this to select the initial therapy of SCCL patients. We are culturing other human tumor cells in this assay and testing their drug sensitivity as well.

Dr. Carney and Dr. Minna

Besides anchorage independence (growth in semi-solid medium) another property of tumor cells that can be exploited is their ability to grow in low concentrations of serum. Because we have defined the SHITE formula of serum free hormone supplemented medium for SCCL we have been systematically transplanting tumors directly from patients into this defined medium. We have found that only the tumor cells survive in this medium, and not normal cells, and that the tumor cells replicate. Infact, in 2-3 days we can greatly enrich for tumor cells and greatly expand the number of TCFU's by growth in the SHITE medium. The SHITE medium appears selective for SCCL and not for other tumor types. We are thus using this selection procedure to develop an entirely different liquid medium assay (Carney-Minna assay) that we feel will

supplant the agar colony forming assay. Tumor cells are plated directly in selective medium into 96 well microtiter plates. Drugs are added by means of a replicator to all 96 wells simultaneously, and then growth is scored by a variety of radioautographic means, but most specifically by using a monoclonal anti-human antibody. The cells are fixed to the plate, and then the amount of antibody bound is proportional to the number of cells. This assay should permit simultaneous testing of a large number of drugs, or drug combinations on a relatively few human tumor cells with great ease. We plan to correlate this with the Hamburger Salmon system.

Dr. Minna, Dr. Cutitta, Dr. Abrams, Dr. Radice, and Mr. Fedorko

We have established the technique of monoclonal antibody production by somatic cell hybrids (hybridomas) and are using it to search for monoclonal antibodies with specificity for lung cancer cells. The test system is to immunize various strains of mice, create hybridomas and the screen using a solid phase radiobinding assays in flexible microtiter plates, followed by radioautography, or ELISA systems on a variety of target cells. Specifically we are focusing on monoclonal antibodies that will bind to tumor cells but not autologous B-cell lines from the same patient. We have screened nearly 25,000 such cultures and have succeeded in producing monoclonal antibodies with specificity for SCCL and for adenocarcinoma of the lung. This feat, was of great effort and required the development of replicators, and large scale screening techniques, to detect the stabilized producers of the desired antibody. However, these same techniques will be used in screening the human tumor cells for drug sensitivity as described in the preceeding section. We plan to continue to isolate these antibodies, grow them up in large quantity as ascites in pristane primed mice, and to characterize the antigenic determinants, and molecules they bind to. A major focus will be on using them to examine clinical specimens. Ultimately such specific antibodies will be of use in early diagnosis, staging and follow-up of patients, may be used for immunotreatment of autologous bone marrow to remove tumor cells, and may have drugs conjugated to them as new modes of therapy. In addition, they will allow a study of the differentiation of the lung cancer cells and the degree of antigenic relatedness between different lung cancer histologic types.

Dr. Radice and Dr. Minna

We have also been attempting to produce monoclonal antibodies against peptide hormones secreted by lung cancer cells including ACTH, endorphine, enkephlins, AVP, calcitonin, bombesin, substance P, and somatostatin. We have immunized various mouse strains with single and multiple combinations of hormones, and then screened for individual hybridoma specificities in the microtiter plate assay. We are attempting to identify and stabilize such intersing hybrids. These antibodies would have far reaching uses in biology, medicine, endocrinology, and as potential tumor markers for lung cancer.

Dr. Minna and Mr. Fedorko

We have also produced and characterized monoclonal antibodies to myoglobin. These antibodies, have high affinities, and single site binding (linear scatchard kinetics). These may be clinically useful to detect human myoglobin,

particularly in some tumors, and will be useful to study the evolution and antigenic structure of myoglobin.

Dr. Oie, Dr. Carney, Dr. Gazdar, and Dr. Minna

Because of the need for autologous B-cell lines for monoclonal antibody testing, and retrovirus gene analysis, we have been systematically trying to establish companion B-cell lines by EBV transformation of lymphocytes from patients whose tumors we have in culture. These should be of great value.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03024-11 VAMO														
PERIOD COVERED October 1, 1979 to September 30, 1980																
TITLE OF PROJECT (80 characters or less) Clinical Trials and Other Clinical Investigations																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <u>Senior Staff Oncologists, PI:</u> <table style="width: 100%;"> <tr> <td style="width: 33%;">John D. Minna, M.D.</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">NCI-VA MOB NCI/WVAMC (USPHS)</td> </tr> <tr> <td>Martin H. Cohen, M.D.</td> <td>(Deputy Chief, Clin)</td> <td>NCI-VA MOB NCI/WVAMC</td> </tr> <tr> <td>Paul A. Bunn, M.D.</td> <td></td> <td>NCI-VA MOB NCI/WVAMC (USPHS)</td> </tr> <tr> <td>Daniel C. Ihde, M.D.</td> <td></td> <td>NCI-VA MOB NCI/WVAMC (USPHS)</td> </tr> </table> <u>Associate Senior Staff Oncologist</u> <table style="width: 100%;"> <tr> <td style="width: 60%;">Stanley E. Shackney, M.D.</td> <td style="width: 40%;">CPB/DCT/NCI/NIH (USPHS)</td> </tr> </table>			John D. Minna, M.D.	Chief	NCI-VA MOB NCI/WVAMC (USPHS)	Martin H. Cohen, M.D.	(Deputy Chief, Clin)	NCI-VA MOB NCI/WVAMC	Paul A. Bunn, M.D.		NCI-VA MOB NCI/WVAMC (USPHS)	Daniel C. Ihde, M.D.		NCI-VA MOB NCI/WVAMC (USPHS)	Stanley E. Shackney, M.D.	CPB/DCT/NCI/NIH (USPHS)
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COOPERATING UNITS (if any) See attached sheets.																
LAB/BRANCH NCI-VA Medical Oncology Branch																
SECTION None																
INSTITUTE AND LOCATION Washington, D.C. VA Medical Center COP/DCT/NCI																
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SUMMARY OF WORK (200 words or less - underline keywords) This project studies new methods of evaluating and treating malignant diseases, including: lung cancer (small cell, epidermoid, adenocarcinoma and large cell carcinoma); prostate cancer; Mycosis fungoides-Sezary syndrome (cutaneous T-cell lymphomas); multiple myeloma; hepatocellular cancer, gastric; and colon carcinoma. Therapies used in phase II and III trials include: intensive combination chemotherapy; combined chemotherapy and radiotherapy (combined modality therapy); (with the Radiation Oncology Branch) and autologous bone marrow transplantation (with the Pediatric Oncology Branch). New chemotherapeutic and immunotherapeutic agents are tested in phase I trials (with the Clinical Pharmacology Branch). Clinical histopathologic correlations are made by the resident pathologists; and extensive pre and post treatment staging of patients to determine the natural history of disease are carried out using: fiberoptic bronchoscopy; peritoneoscopy; CAT scans, and the study of tumor markers by immunoassay (in collaboration with the Frederick Cancer Center). Studies of paraneoplastic syndromes, complications of therapy, nutritional derangements; and second malignancies are also carried out. Clinical associates are trained in medical and oncology and clinical investigation.																

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2.0 CLINICAL TREATMENT STUDIES

2.1 Small Cell Carcinoma of the Lung (SCCL).

2.101 Randomized trial of intensive combination chemotherapy plus and minus simultaneous chest radiotherapy in limited stage SCCL. (CMC+ Chest RT + VAP) (NCI-VA Protocol 77-4; Dr. Cohen and Senior Staff in collaboration with the Radiation Oncology Branch, Dr. A. Lichter and the Biometry Branch, Dr. R. Makuch).

The question asked in this study is whether thoracic radiation therapy initiated on day 1 of chemotherapy will increase survival of limited stage small cell lung cancer patients treated with intensive combination chemotherapy. The NCI-VA prior protocol (75-4) showed that with chemotherapy alone (CMC, VAP) that 70% of limited stage SCCL patients could achieve a complete remission (CR) and 10-20% of these could achieve long term disease free survival including, in some cases, potential cure. A prior study of ROB DCT/NCI/NIH used simultaneous chemotherapy (vincristine, adriamycin, cyclophosphamide) and chest radiotherapy reported even higher long term disease-free survivals but with substantial toxicity. The 77-4 study asks in a prospective randomized fashion whether the two modalities given simultaneously are better in terms of complete response rate, long term disease free survival and toxicity (acute and chronic) than chemotherapy given alone. All patients receive cyclic alternating CMC-VAP chemotherapy (cyclophosphamide, methotrexate, CCNU; vincristine, adriamycin, procarbazine). Patients are all simulated for chest radiotherapy and then randomized to receive 4000 rads/15 fractions/3 weeks or no radiotherapy concurrent with CMC chemotherapy. The esophagus and spinal cord are shielded for the last 1000 rds. Prophylactic brain radiation is given to all patients during weeks 23 and 24.

Thirty-two patients have been entered, 16 on each arm. Groups are comparable as regards age, sex and performance status. The complete response rate for chemotherapy alone is 40% versus 71% for combined modality patients ($P < .20$). There have been 3 early deaths in the chemotherapy-radiation therapy arm. All of these deaths were from pulmonary insufficiency following the acute appearance of diffuse interstitial infiltrates on chest x-ray. All deaths occurred while a full 4000 r was given via opposed PA and AP ports. Since posterior shielding of the spinal cord and esophagus were introduced, there have been no toxic deaths. The combined modality approach has provided better control of the primary tumor than has chemotherapy alone. Considering only complete responders to treatment there have been 67% relapses in the primary tumor areas in chemotherapy alone patients versus 10% in combined modality patients. Thus far, 25% of combined modality patients are disease-free beyond 2 years versus 8% of chemotherapy alone patients. The study is ongoing.

2.102 Intensive combination chemotherapy with cyclophosphamide, adriamycin, VP-16-213, and Oncovin (Vincristine) "CAPO" in extensive stage SCCL. (NCI-VA Protocol 77-3; Dr. Minna and Senior Staff).

The purpose of this protocol was to test the effect of giving a large number of high dose combination chemotherapy cycles during 6-8 weeks of induction therapy to previously untreated extensive stage SCCL patients and to compare the results to our prior trial that used one course of intense cyclophosphamide, methotrexate, CCNU therapy followed by moderate doses of the non-cross resistant combination of vincristine, adriamycin and procarbazine (CMC-VAP). We compared the regimens for initial complete response rate, survival and treatment related toxicity. We had concluded from previous trials that a complete response to initial therapy is an important predictor of survival and possible cure; that high dose induction therapy was better than moderate dose therapy for achieving a complete response; that adding new drug combinations after the initial induction treatment gained more complete responders but these were less durable than complete responses obtained early; and that increasing the dose of one of the drugs, cyclophosphamide above 3-4 grams during 6 weeks of induction had no dramatic effect on complete response rate or survival.

Patients had extensive pretreatment staging procedures including biopsy of bronchus, liver, and bone marrow, and peritoneoscopy with multiple liver biopsies if the percutaneous biopsy was negative. In addition the usual radionuclide scan of brain, liver and bone were obtained 12 weeks after the start of therapy patients were re-staged for degree of tumor response. The initial induction therapy consisted of CAPO and groups of patients were given either 5-6, 3-4, or 2 cycles of the combination chemotherapy during the 6-8 weeks of induction. The patients were not randomized to these groups but were allocated to treatment by time of entry onto protocol; the 5-6 cycle group was treated first; the next group received 3-4 cycles, and the final group 2 cycles of induction therapy. This decrease was necessitated by the toxicity of the regimens with the increased number of cycles. Thus, in addition to comparing the results to the CMC-VAP trial, the 2 cycle group was compared to patients receiving more than this number of cycles. Following induction therapy, the patients were maintained on outpatient chemotherapy using the same drugs every three weeks with dose modifications for hematologic toxicity. Prophylactic cranial irradiation was given at week 23, and all patients with overt brain metastases were given therapeutic brain irradiation.

50 patients entered the study between November 1977 and November 1979. The median length of follow-up is 19 months with a follow-up range of 7-24 months. 34 patients were treated with 3-6 cycles (11 received 5-6, and 23, 3-4 cycles) for a mean of 4-5 cycles of induction therapy. 16 patients were treated with 2 induction cycles. The groups and the prior CMC-VAP trial patients were similar in

respect to initial performance status and other prognostic variables. All of the patients who received 3-6 cycles of induction therapy had objective tumor regression while only 63% of the group receiving 2 induction cycles responded. When strict criteria for a complete response are used, the complete response rate for the 5-6 cycles group was 27%, for the 3-4 cycle group 26% but for the 2 cycle group only 13%. These were all less than the 36% complete response rate seen with CMC-VAP. Initially it was planned to treat all patients with 6 cycles of therapy, but toxicity including severe granulocytopenia, septicemia, weight loss, and marked decrease in performance status prevented this and required cutting back on the number of induction cycles. The percentage of bacteremic patients was 22% for the 3-6 cycle group and 24% for the 2 cycle group, both slightly higher than the 15% seen with CMC-VAP. However, the deaths related to treatment toxicity in the first two months were 18% in the 3-6 cycle group, 6% in the 2 cycle group and 7% in the CMC-VAP group.

Actuarial survival curves of the 3-4, 5-6, 2 cycle and CMC-VAP groups were not significantly different. In addition, the survival curves of the complete responders were also not different. Finally, it appears only 2 patients will be long term disease free survivors in this trial, not different from that seen with the CMC-VAP treatment in extensive stage disease.

We conclude from these studies that in extensive stage SCCL that: First, the complete response rate, duration of complete response or survival are not increased by giving a very intensive 3-6 cycle regimen of cyclophosphamide, adriamycin, VP-16, and vincristine compared to prior trials with a less intense regimen of CMC-VAP trial. A steep dose response curve exists in SCCL such that 2 cycles of the same therapy gives fewer objective and complete tumor responses than does the 4 or more cycle regimen. This result is consistent with our prior randomized trial showing a decrease in the complete response rate with less intensive therapy. Finally, we conclude that other methods besides increasing the initial intensity of induction therapy either by dose or number of induction cycles of the same combination need to be considered in the treatment of extensive stage SCCL.

2.103 Combination chemotherapy and chest radiotherapy at week 12 for SCCL. (CMC, VAP, chest RT)(NCI-VA Protocol 77-1; Dr. Cohen and Senior Staff in collaboration with the WVAMC Radiotherapy Service.)

Following NCI-VA protocol 75-4 (CMC, VAP) it was noted that some patients not in a complete response at the end of CMC would enter a complete response if treated with VAP. This study (77.1) was designed to ask whether thoracic radiation therapy given after CMC and VAP (week 12) would induce additional patients to enter a complete remission and prolong the overall and disease free survival of patients already

in a complete remission as compared to historical controls treated with CMC-VAP alone. Radiation therapy was given as 2000 rads/ 5 fractions/one week to the primary tumor site and to mediastinal lymph nodes.

Sixteen patients with SCCL were entered. Two of the 16 remained disease-free for 24+ months. Both of these patients presented with limited stage disease. It is clear that thoracic radiation therapy as given, was of no benefit to patients with extensive stage disease compared to the historical controls and no further patients entered a complete remission. Patient numbers are too small for firm conclusions as to the efficacy of radiation therapy given this way in limited stage disease. Toxicity of this treatment program was acceptable, although one patient developed reversible bilateral pulmonary interstitial disease. This study is terminated.

2.104 Randomized trials of thymosin therapy in SCCL. (NCI-VA Protocol 76-4, 77-1, 77-3, 77-4; Dr. Cohen and Senior Staff, in collaboration with Dr. Chretien, Surgery Branch and Dr. Makuch, Biometry Branch.

These protocols asked in a prospective randomized fashion whether calf thymosin, fraction V, influences survival of patients with SCCL. Patients were randomized to receive thymosin fraction V (Hoffman La-Roche) 60 mg/M² thymosin 20 mg/m² or no thymosin treatment. The former two groups received thymosin twice weekly for the initial six weeks of induction chemotherapy. After 55 protocol eligible patients had been entered the thymosin 20 mg/m² dose was dropped and the remaining patients received thymosin 60 mg/m² or no thymosin treatment.

Results as of April 1980, from the first 46 protocol eligible patients treated on protocol NCI-VA 75-4 indicated that thymosin 60 mg/m² significantly prolonged survival of treated patients as compared to thymosin 20 mg/m² or no thymosin treatment. (P = .047). Survival gain was due to prolongation of the duration of complete response. There was no effect of thymosin on the complete response rate or on infectious episodes during remission induction. Patients presenting with low total peripheral blood lymphocyte counts derived

greater benefit from thymosin than individuals with normal blood lymphocyte counts. Clinical results have been published in J^A 241: 1813, 1979 and laboratory correlations have been published in Treatment Reports 62: 1787, 1978

Currently 1 of these 46 patients remains alive and disease free 4 years after initiation of treatment.

Subsequently an additional 21 patients, treated on 3 different therapy protocols were randomized to receive thymosin 60, 20 or 0 mg/m². When thymosin 20 was found to be a therapeutically ineffective dose, this was dropped. After statistical correction for imbalances in prognostic factors among the treatment groups among the total 67 patients, the T60 group had a significantly superior survival experience compared to T20 or T0 patients ($p > .60$) while survival for patients in the T60 group was superior in comparison to either the T0 or T20 patients ($p = .008$, $p < .001$, respectively).

Currently 5 of the 67 patients are disease-free 2 or more years from onset of therapy. Four of these patients receive T60. The fifth patient had limited disease and received concomittant chemotherapy-radiation therapy.

Thymosin shipments from Hoffman-LaRoche were discontinued for unclear reasons in February 1978 and no patients have been entered since then. Current studies utilize a human thymosin fraction prepared by Syntex Research

Thymosin Studies of SR-270258 in Solid Tumor Patients:
A study of the tolerance and biological activity. (NCI-VA MOB 79-5: Dr. Cohen and Senior Staff).

A protein fraction with thymic hormone-like activity (SR-270258, Syntex Research, Pal Alto, California) has been isolated from human blood. This substance, which is present in the prealbumin fraction of human blood has biological activity in in vitro model systems similar to other thymic hormone-like substances derived from animal sources. A phase I study is planned, in previously untreated solid tumor patients to determine the toxicity of this material and to determine activity as measured by several in vitro immunologic assays, by delayed hypersensitivity, skin testing and by serum immunoglobulin determinations.

Two patients have been entered, todate at 1 mg/m². There has been no toxicity. The study is ongoing.

2.105 Phase II Trial of Doxorubicin, Mitomycin-C in advanced non-small cell lung cancer. (NCI-VA MOB Protocol 78-4; Dr. Cohen and Senior Staff).

This study continues efforts to define an active cytotoxic drug regimen for non-small cell lung cancer. Mitomycin-C is receiving new attention as an active agent in this disease. In the current study it has been combined with doxorubicin and administered in three week cycles to ambulatory patients with advanced Stage III or Stage IV non-small cell lung cancer.

Twenty-two patients have been entered on study, 4 with squamous cell, 1 with large cell, and 17 with adenocarcinoma of the lung. Major responses have been observed in 9 of 17 patients with adenocarcinoma (1 complete, 8 partial) but in 0 of 5 patients with other cell types.

Three patients have died of treatment-related complications, one at six weeks of sepsis associated with leukopenia, one four months of interstitial pneumonitis attributed to chemotherapy and another at six weeks of similar pulmonary toxicity. The latter patient had received prior radiation therapy to the lung and developed toxicity after only two reduced doses of drug. An additional 3 patients have also developed diffuse interstitial infiltrates on chest x-ray but have survived this complication. Because of the demonstrated activity of the adriamycin-mitomycin C combination and because of the high frequency of lung toxicity (23%). The mitomycin C schedule was changed so that this drug was administered every 6 weeks. Ten patients have been entered on this new schedule (6 adeno, 2 squamous, 2 large cell). Eight patients are currently evaluable for response. There has been one partial response and 6 patients have stable disease.

2.106 Phase II trial of Cis-platinum in patients with SCCL failing primary therapy. (NCI-VA protocol 77-7; Dr. Levenson, Dr. Ihde and Senior Staff).

This trial was designed to determine if cis-platinum (CDDP) could achieve objective responses in patients with SCCL failing prior therapy and to determine the degree of hematologic toxicity. 14 patients have been entered, and 2 minor responses have been seen. Patients have been treated with 24 hour infusions at doses of 100 mg/M² every 3 weeks. The most significant toxicity noted was severe thrombocytopenia found in 2 patients. We conclude that cis-platinum has minimal activity in SCCL patients failing prior combination chemotherapy, and these patients can experience moderate to severe hematologic toxicity.

2.107 Phase II trial of hemibody radiotherapy with and without radio-protector WR-2721 in patients with SCCL failing primary or secondary chemotherapy (NCI-VA protocol 77-2; Dr. Ihde and Senior Staff in collaboration with Drs. Glatstein and Schwade, ROB).

SCCL is highly responsive to radiotherapy at the time of diagnosis. We are investigating the antitumor effect of this modality given as a more systemic treatment (hemibody radiotherapy) in previously unirradiated SCCL patients who have failed combination chemotherapy programs. A treatment schedule of 400 rads per week to the upper half-body x 4 (total dose 1600) rads was planned. However, in contrast to previously reported results from Canadian patients who had generally not been exposed to chemotherapeutic agents, hematologic toxicity prevented all but one of five patients treated from completing the planned course of therapy. A sixth patient was treated with 200 rads twice in 1 week and then had treatment discontinued because of peripheral blood count depression. In 2/6 cases, thrombocytopenia was profound and required platelet transfusions. Objective tumor regression (not fulfilling the standard definition of a partial response) was observed in 2 patients. We conclude that fractionated hemibody irradiation produced substantial hematologic toxicity in SCCL patients previously receiving intensive combination chemotherapy, and a suitable treatment schedule for this patient population has yet to be developed. We currently plan to treat additional patients with 200-400 rads per week in conjunction with administration of the radioprotective agent WR-2721.

This drug is highly effective in ameliorating the toxicity of whole body radiotherapy in animals, Phase I trials in humans are presently underway elsewhere. Upon their completion, a suitable dose to be used in our trial should be known.

2.108 Evaluation of radiotherapy in control of local disease in SCCL (Dr. Ihde and Senior Staff, in collaboration with Dr. Lichter and Senior Staff, ROB).

We have established that chest irradiation in the usual palliative doses is ineffective in providing long-term local control of small cell lung cancer that is progressive despite the administration of chemotherapeutic agents (*Radiology* 132:443, 1979). Therefore, when our patients require radiotherapy for local palliation after failing chemotherapy in the future, higher doses than the previously utilized median of 3200 rads in 10 fractions will be administered. All patients are given 4000 rad in 10 fractions in a split course. Actuarial duration of local freedom from disease progression will be computed under this new policy. It is hoped that it will be superior to our previous results of 50% progression at 3 months and 90% progression at 12 months.

2.109 Intensive combined modality (chemotherapy plus multiple field local irradiation) therapy followed by autologous bone marrow hematopoietic reconstitution for patients with extensive stage SCCL in complete or partial remission (NCI-VA Protocol 80.3; Dr. Ihde and Senior Staff, in collaboration with Drs. Deisseroth and Abrams, POB, Drs. Lichter and Glatstein, ROB, and Dr. Makuch, BRB).

This protocol was recently approved by the NCI Clinical Research Subpanel, and patients will begin to be eligible for accrual in June, 1980. Although a substantial fraction of SCCL patients with extensive disease achieve complete or partial remission with combination chemotherapy (80-95% in our hands), most will eventually relapse, and long-term disease-free survival even in complete responders is less than 10%. Three observations suggest that this patient population is an ideal one in which to attempt to induce additional or prolong existing complete remission with high-dose combined modality therapy: 1) higher doses of chemotherapy produce superior responses in this disease; 2) ablative therapy, which autologous hematopoietic reconstitution permits, induces some complete responses in SCCL patients who have progressive tumor after primary therapy; and 3) in acute myelogenous leukemia, such intensive therapy is much more successful in patients in complete remission than in those with relapsing disease, and has resulted in dramatic increases in disease-free survival.

The outline of the protocol is as follows: Patients will undergo our standard 6-week induction chemotherapy program of cyclophosphamide, methotrexate, and CCNU; six weeks of outpatient therapy with vincristine, adriamycin and procarbazine will then be administered. At Week 13, extensive re-staging procedures will be performed to ascertain if the patient is in complete or partial response. Autologous bone marrow will be collected from all responding patients without bone marrow involvement by standard examination or by the soft agar clonogenic assay. Late intensive therapy with multiple field local irradiation (2000 rad/5 fractions/5days) to all sites initially involved with tumor will be followed by three days of high-dose chemotherapy with Cyclophosphamide, to which all patients will have previously responded

and with VP-16, an active agent to which the patients' tumors will not have been exposed. Autologous cryopreserved marrow will then be administered. After recovery of hematopoietic function, patients in complete response will be followed without further antineoplastic therapy except for prophylactic cranial irradiation. It is uncertain whether autologous hematopoietic reconstitution is absolutely required in order to administer this late intensive therapy, but since most or all patients would be expected to have minimal symptoms from their tumor at the time of therapy it is considered important to reduce potential risks of the treatment as much as possible. It is anticipated that 30 patients with extensive SCCL will begin induction chemotherapy in the next two years, of whom half might be eligible for the late intensive therapy. These patient numbers will allow detection of a three-fold increase in 2-year survival compared to our historical controls.

2.110 Intensive combined modality therapy with autologous peripheral blood buffy coat infusion in patients with extensive stage SCCL in partial remission after initial chemotherapy (NCI-VA Protocol 79-4; Dr. Ihde and Senior Staff in collaboration with Drs. Abrams and Deisseroth, POB, Drs. Lichter and Glatstein, ROB and Dr. Makuch, BRB).

Survival is substantially prolonged in SCCL patients who achieve a complete response with combination chemotherapy. For partial responders, however, survival is only moderately longer than in patients without response. These observations raise the question of whether partial responders after initial treatment should be subjected to more aggressive therapy in an attempt to induce a complete response. Two patients with extensive SCCL had peripheral blood buffy coats collected during recovery from CAPO (cyclophosphamide, adriamycin, VP-16, and vincristine) induction chemotherapy. Dr. Abrams has shown that the number of circulating CFU_c's (the putative myelocytic stem cells) markedly increase after intensive chemotherapy, and that infusion of peripheral blood buffy coat collected under similar circumstances in dogs can prevent lethality from whole body irradiation. The patients received local irradiation (2000 rad/5 fractions/5 days to their chest tumor (which had not resolved completely on CAPO), followed by high doses of cyclophosphamide and VP-16 and then autologous buffy coat infusion. One patient required 35 days for hematologic recovery and did not enter complete remission; the other recovered in 25 days and did achieve complete response. This protocol has been discontinued because of the conclusion of CAPO induction therapy; both complete and partial responders will now be entered on Protocol 80-3 (Sect. 2.109).

2.111 Reviews of therapeutic results, staging procedures and prognostic factors in SCCL (Drs. Ihde and Bunn, in collaboration with Dr. Hansen, Fnsen Institute)

Literature reviews of current therapeutic results in SCCL, combination chemotherapy of SCCL, and staging and prognostic factors in SCCL have recently been completed and are in press.

2.2 Non-small Cell Carcinoma of the Lung (NSCCL) including: Adenocarcinoma, Epidermoid Carcinoma, Large Cell Carcinoma, and Mixed Histology. (Small Cell/Large Cell) Carcinoma.

2.201 Treatment of Non-small cell carcinoma of the lung (NSCCL) with high dose methotrexate by 6 or 30 hour infusion followed by leukovorin factor rescue. (HDMTX) (NCI-VA Protocol 76-9; Dr. Minna and Senior Staff).

Because of prior reports of efficacy of HDMTX in cancer we have completed a phase II trial of this modality which is being prepared for publication. 14 patients were treated by 6 hour infusion (in doses up to 12 gm/M^2) and 18 patients were treated by 30 hour infusion (1.5 gm/M^2) followed by leucovorin rescue. No responses were seen in the 6 hour infusion; 3 responses (17%) were seen in the 30 hour infusion protocol. No renal toxicity, or hematologic toxicity was encountered. The MTX levels were monitored by a radiobinding assay. MTX levels of 4×10^{-3} were seen with the 6 hour infusion protocol. We conclude that HDMTX with leucovorin rescue has no increase in activity over that reported for standard dose MTX in NSCCL and that it may be given with safety. Although the comparison of the 6 and 30 hour schedules was done sequentially and not in randomized trials the occurrence of all responses in the 30 hour protocol suggests that if HDMTX is to be used in further studies the 30 hour regimen should be considered.

2.202 Phase II Trial of Doxorubicin, Mitomycin-C in advanced non-small cell lung cancer (NCI-VA MOB Protocol 78-4; (Dr. West and Senior Staff

This study continues efforts to define an active cytotoxic drug regimen for non-small cell lung cancer. Mitomycin-C is receiving new attention as an active agent in this disease. In the current study it has been combined with doxorubicin and administered in three-week cycles to ambulatory patients with advanced Stage III or Stage IV non-small cell lung cancer.

Twenty patients have been entered on study, 4 with squamous cell, 1 with large cell, and 15 with adenocarcinoma of the lung. Major responses have been observed in 8 of 10 patients with adenocarcinoma but in 0 of 5 patients with other cell types (5 additional patients with adenocarcinoma have received on cycle only and are inevaluable for response.

Three patients have died of treatment-related complications, one at six weeks of sepsis associated with leukopenia, one at four months of interstitial pneumonitis attributed to chemotherapy and another at six weeks of similar pulmonary toxicity. The latter patient had received

prior radiation therapy to the lung and developed toxicity after only two doses of drug. Prior radiation therapy to the lung is now an absolute exclusion for entry to study, and patients are being evaluated closely for evidence of pulmonary toxicity. We conclude that the combination of adriamycin and mitomycin-C in this schedule is highly active in adenocarcinoma of the lung. The incidence of pulmonary toxicity must be carefully monitored.

2.203 Combination Chemotherapy of mixed histology Large Cell: Small Cell Lung Cancer (WPL #40/22). (Various NCI-VA Protocols including 5-4, 77-1, Dr. Makuch, Biometry Branch).

Twenty-three previously untreated patients with lung cancer were found to have large cell carcinoma (WPL #40) mixed with intermediate subtype small cell carcinoma (WPL #22) within the same lesion at presentation. These patients were treated with intensive combination chemotherapy (CMC VAP, or CMC). There were two complete remissions (CR) (13%), and 10 partial responses (PR) (43%). The total objective response rate of 56% was lower than the 89% objective response rate in similarly treated group of 59 patients of 24%. The overall survival and time to progression after initial therapy were significantly shorter in the mixed histology patients than in those with pure SCCL. Autopsy findings revealed no single consistent histologic subtype post therapy. We conclude that mixed histology large cell/small cell lung cancer (WPL #40/22) is a distinct entity that 1) can be misdiagnosed as large cell cancer; 2) has a better total response rate than pure large cell cancer (WPL #40); but 3) has a low CR when compared to pure SCCL (WPL #21, 22, 21/22). Thus, for the purposes of treatment and investigation, this entity must be distinguished from other lung cancer subtypes with differing responses to therapy and may account for the high response rates in other series of large cell carcinoma.

2.3 Gastric Carcinoma. Phase II trial of ICRF 159 in gastric carcinoma (NCI-VA Protocol 77-17; (Dr. Bunn and Senior Staff).

18 patients have been entered on this trial including 11 previously untreated patients and 7 patients who had previously failed the MF-AM protocol (NCI-VA Protocol 76-8). Only one of the previously untreated patients has responded and one of the 7 previously treated patients have responded for a total of 2 responses in 18 patients. The two responders had a remission duration shorter than two months. Toxicity was limited to mild hematologic suppression. Approximately 5 more previously untreated patients will be entered onto this study. At this time it appears that ICRF-159 has very limited activity in gastric carcinoma and appears to be less active than either 5-FU, Adriamycin and possibly the nitroureas. This is the only Phase II single agent trial of ICRF-159 in gastric cancer that we are aware of. Combination chemotherapy trials conducted by the GI Tumor Study Group have also suggested that ICRF-159 has little or no activity in gastric cancer.

2.4 Prostatic Carcinoma

2.401 Combination chemotherapy with cyclophosphamide, adriamycin, and cis-Platinum followed by hormonal therapy for patients with previous untreated disseminated extrapelvic carcinoma of the prostate (NCI-VA Protocol 77-8; Dr. Ihde and Senior Staff in collaboration with Dr. Lippman, MB, Dr. Javadpour, SB, Dr. Bensimon, Urology Svc., WVAMC, and Dr. Dunnick, Diagnostic Radiology, CC).

We have previously demonstrated (Cancer 45:1300, 1980) that combination chemotherapy can produce objective tumor responses associated with prolonged survival in patients with extrapelvic metastatic prostatic cancer resistant to hormonal therapy; furthermore, with the use of extensive serial staging procedures, both tumor response and progression can be objectively documented, usually in multiple ways in each patient. We have now instituted a trial of combination chemotherapy with cyclophosphamide, adriamycin, and cis-Platinum in recently diagnosed patients with metastatic disease who have not received previous hormonal treatment. At the time of disease progression, patients receive an orchiectomy. Before initiating the protocol, percutaneous prostatic biopsies are obtained for determination of sex steroid receptor proteins. Results of receptor determinations will be correlated with response to each form of therapy. This trial should provide information on whether chemotherapy can successfully be given to freshly diagnosed patients, whether it may have a higher objective response rate than in patients who have already failed hormone therapy and whether patients who have failed chemotherapy retain the expected high rate of responsiveness to hormone manipulation. If the response rates to both chemotherapy and orchiectomy are adequate, a randomized trial of chemotherapy as an adjuvant to initial hormone treatment is planned. Thus far, 14 patients have entered the trial. There are 4 partial responses, 3 stable disease patients, 4 patients with progressive disease after 4 cycles of chemotherapy and 2 who are not evaluable.

2.402 Combination chemotherapy with cyclophosphamide, adriamycin and cis-Platinum for hormone-resistant prostatic carcinoma (NCI-VA Protocol 77-8; Dr. Ihde and Senior Staff, in collaboration with Dr. Lippman, MB, Dr. Javadpour, SB, Dr. Bensimon, SVAMC, and Dr. Dunnick, DR/CC).

The combination chemotherapy program used in patients with newly diagnosed metastatic prostatic cancer will also be given to patients who have failed hormone manipulation. This should provide some estimate of the program's relative efficacy in the two patient populations. Prostatic biopsies for steroid receptor determinations will also be done in these patients. Thus far, 5 patients have been entered on study. One had stable disease, 3 had progressive disease, and one is too early to evaluate.

2.5 Cutaneous T-Cell Lymphomas: Mycosis fungoides and the Sezary Syndrome (MF-SS).

2.501 Combined modality treatment with electron beam radiation and chemotherapy in the treatment of cutaneous T-cell lymphomas (NCI-VA Protocol 76-12; Dr. Bunn and Senior Staff in collaboration with Dr. Schechter, Hematology VAMC; Dr. Fischmann, Dermatology VAMC, Dr. Goldson, Radiotherapy, Howard University, Dr. Glatstein and Senior Staff, Radiation Oncology Branch).

Combined modality electron beam and intravenous Meclorothamine for plaque stage cutaneous T-cell lymphoma (Stage IA and IB). In this trial 14 patients were entered; the trial is now closed to patient entry. Preliminary results of this trial were published in Cancer Treatment Reports and were presented this year at the annual meeting of the American Society of Clinical Oncology. Ten of the 14 Stage I patients achieved a complete remission and the other 4 patients achieved a partial remission. The complete responders received 3 monthly cycles of intravenous mechlorethamine and have been in unmaintained remission since the completion of therapy. The minimal follow-up is 17 months with a maximum follow up of 38 months. None of the complete responders have relapsed to date. The four partial responders were treated with topical nitrogen mustard and none have noted progressive disease over the follow up period. Only one of these patients has died and he died a sudden death in complete remission unrelated to tumor or therapy toxicity. Little toxicity was noted. There was mild myelosuppression from the mechlorethamine. One patient had a platelet count below 100,000. The results from Stanford University using electron beam therapy along as published in Cancer Treatment Reports in 1979 also reveal overall excellent survival. In both studies 3 years survival approximated 90 per cent but it was slightly better in our trial. Actuarial disease free survival at three years was 71% in our series and 39% in the Stanford series. Our conclusion from this study was that encouraging results were obtained in these Stage I patients. These results must be tempered by the fact that median survival in Stage I patients treated with electron beam therapy or topical nitrogen mustard alone approaches 8 to 9 years. Thus, a randomized trial comparing combined modality therapy with less intensive approach is needed. Such a trial is now in progress at the National Cancer Institute (See below).

2.502 Combination chemotherapy vinblastine, adriamycin, bleomycin, VAB alternating with cyclophosphamide, methotrexate and prednisone (CMP) and simultaneous electron beam radiotherapy for cutaneous T-cell lymphomas (NCI-VA Protocol 76-12).

25 patients were entered on this study which is now closed for patient accrual. Preliminary results were published in Cancer Treatment Reports and data from this study was presented at the annual meeting of the American Society of Clinical Oncology in May 1980. Minimum follow up is 17 months, maximum follow-up 38 months and a median follow-up of 32 months. Four of 11 patients in Stage II and Stage III achieved a complete remission and 3 of 14 Stage IV patients achieved a complete remission. Two of the 4 Stage II and III complete responders have relapsed and all 3 of the Stage IV complete responders have relapsed. The disease free survival for patients with these stages was significantly inferiorly to that achieved in Stage I. Only 1 patient had no response and 17 of the 25 patients had a partial response. The median duration of partial response was significantly shorter than median duration of complete response. Median survival for this group of patients will exceed three years. However, of Stage IV patients, survival and 10 of the 14 patients have died with an actuarial median survival of 34 months. Comparison of the survival and disease free survival in this study with those recently published in Cancer Treatment Reports in 1979, from Stanford University using electron beam therapy alone show minimal benefit for the combined modality approach. Only about 8 per cent of patients are disease free for more than 3 years. Toxicity in this trial was again primarily cutaneous. Approximately two-thirds of the patients had significant cutaneous toxicity. Three patients have developed second skin cancers. Five male patients have developed gynecomastia. Myelosuppression due to the chemotherapy has been extremely mild. There have been no deaths from toxicity in this trial. Our conclusions from this study were that combined modality therapy is highly effective in producing objective tumor remission. However, a minority of the patients entered a histologically documented complete remission. While combined modality therapy can be given safely, cutaneous toxicities are frequent and extreme attention to detail must be given when delivering electron beam irradiation with or without chemotherapy. The degree of response duration and survival are directly related to the initial disease stage. The cutaneous T-cell lymphoma staging classification proposed at the national workshop under the direction of Dr. Bunn appears to be useful in defining prognostic groups. While useful palliative responses were seen in 96 per cent of patients with advanced disease only 8 per cent are currently disease free, meaning the combined modality approach has failed to produce a large fraction of durable complete remissions in advanced stage patients. This finding is similar to the results in adult advanced stage adult B cell lymphomas. Thus, we feel that further aggressive combined modality studies should be compared in a randomized trial to more conservative measures in this chronic disease. Such a randomized trial is in progress in our branch (see below).

2.503 Randomized trial of conservative versus combined modality therapy for cutaneous T-cell lymphomas (NCI-VA Protocol 79-2; (Dr. Bunn and Senior Staff in collaboration with Dr. Schechter, Hematology VAMC; Dr. Fischmann, Dermatology, VAMC; Dr. Golson, Radiotherapy, Howard University; Dr. Glatstein and Senior Staff ROB; Dr. Edwards, Biometry Branch).

This protocol was designed to determine whether combined modality therapy with electron beam irradiation and combination chemotherapy with cyclophosphamide, adriamycin, VP-16 and vincristine ("CAPO") with curative intent is better than standard sequential topical therapies including topical nitrogen mustard, photochemotherapy with methoxsalen and ultraviolet A light (PUVA), and electron beam therapy with palliative intent. This protocol has been approved and instituted. Since the onset of this protocol all radiotherapy is currently being given at the Radiation Oncology Branch of the National Cancer Institute under the direction of Dr. Glatstein. To date 23 patients have been entered on this trial, 12 of whom have randomized to combined modality treatment and 11 of whom have randomized to conservative topical treatments. It is currently too early to evaluate the results of this trial. It is expected that there will be a minimum of 100 patients entered on this trial. At the present rate accrual of these 100 patients will take about 4 or 5 years as initially projected. To date toxicity has been acceptable on this protocol and there have been no deaths from toxicity. There has been one death in the study from progressive disease which occurred in a patient who was randomized to combined modality treatment.

2.504 The branch has been actively involved in literature reviews in the treatment of the cutaneous T-cell lymphomas and several of these reviews have been published during the year.

2.505 Phase II trial of antithymocyteglobulin (ATG) in the treatment of cutaneous T-cell lymphomas. This study is being conducted in cooperation with the Upjohn Company from Kalamazoo, Michigan and involves the intravenous administration of ATG to patients with advanced cutaneous T-cell lymphomas who have failed previous chemotherapy and topical therapies. Seven patients have been entered on this trial, however; only four of these patients are evaluable for response. One patient who developed serum sickness after several days of treatment and two patients who died shortly after the treatments are also inevaluable for response. Three of the remaining institution of four patients had a partial remission which lasted from 1 to 8 months, one patient had no response. Toxicity from this treatment has been considerable. Fevers and shaking chills follow the administration of the ATG. In addition, at least two of the

patients have developed leukopenia and thrombocytopenia. Two of the patients who died early in the trial died from infections which were felt to be from the skin and unrelated to the therapy. However, a role of the therapy in depressing immune response cannot be ruled out. These toxicities have been reported to the FDA and to the Upjohn Company. The preliminary conclusions from this trial are that antithymocyteglobulin is in fact active in this disease. However, toxicity prohibits its continued use. We anticipate entering only three more patients on before terminating the trial.

2.506 Phase II trial of VP16-213 in the therapy of cutaneous T-cell lymphomas. (Dr. Bunn and Senior Staff).

Seven patients have entered this trial, four patients had a partial response and three patients had no response. One patient developed severe myelosuppression of life threatening nature having previously received multiple chemotherapeutic agents. We plan to enter another 7 to 10 patients on this trial which is still open to patient accrual. Preliminary conclusions are that VP-16 does indeed appear to be an active agent in patients failing other chemotherapeutic agents.

2.507 Phase II trial of high dose methotrexate in cutaneous T-cell lymphomas. (Dr. Bunn and Senior Staff).

12 patients were entered on this trial after having previously failed multiple other chemotherapeutic agents. Five of these 12 patients have achieved a partial remission and 7 patients had no response. There were no complete responders. The median duration of remission has been two months and none of the remissions lasted for more than 6 months. No myelosuppression was noted from the treatment nor did renal toxicity or other serious complications occur. Preliminary conclusions from this trial are that high dose methotrexate can be tolerated in patients failing multiple other drugs and has a reasonable response rate. However, the response duration is of limited nature.

2.508 Cytogenetic studies in cutaneous T-cell lymphomas (NCI-VA Protocol 76-12 and 79-2; in collaboration with Dr. Whang-Peng, Medicine Branch).

Cytogenetic analysis is performed on the peripheral blood, lymph nodes and bone marrow of all patients prior to and following therapy. Preliminary results of this work have been published in Cancer Treatment Reports and a definitive article has been submitted to the American Journal of Medicine.

This study showed that the majority of patients (83%) with cutaneous T-cell lymphomas, including untreated patients with early stages of disease, have cytogenetic abnormalities detected in the peripheral blood and lymph nodes. The chromosomal abnormalities were both structural and numerical in type and involved nearly all the chromosomes. These abnormalities may be detected in tissues histologically negative as well as in those histologically positive for tumor by light microscopy. Frequency of abnormal metaphases and the type of cytogenetic abnormalities were related to the stage of disease and to prognosis. Patients with few abnormal metaphases were more likely to have early stages of disease especially with plaque lesions. A higher percentage of abnormal metaphases was associated with advanced stages of erythroderma and prior systemic therapy. Cytogenetic findings gave important prognostic information as patients with frequent abnormal metaphases (especially hyperdiploidy), and clone formation had the most aggressive disease clinically whether or not they had been previously treated. The presence of hyperdiploidy in clone formation was also associated with a large cell histologic variant. This association was present at diagnosis in some patients and was associated with a change of histology from small cell to large cell in patients with progressive disease. Unlike chronic myelogenous leukemia, clone formation occurred in a minority of patients.

In addition, when clone formation occurred not all cells had the clonal marker and clonal chromosome markers varied from patient to patient. The frequent finding of cytogenetic abnormalities in the early stages of disease in histologically negative specimens makes cytogenetics a useful adjunct to other studies in making an early diagnosis. The wide variety of karyotypic abnormalities found in individual patients early in the course of the disease suggests that either the disease is polyclonal or that it is monoclonal with great chromosomal instability. Clonal abnormalities develop only late in the disease and appear to represent the selection of a group of cells with proliferative advantage. Our studies suggest that these clones usually have increased chromosome numbers and proliferate more rapidly.

2.509 Studies of Viral Oncogenesis in the Cutaneous T-Cell Lymphomas (Dr. Bunn and Senior Staff in collaboration with Drs. Gallo, Ruscetti and Poesz).

We have shown that a putative type C virus can be isolated from patients with cutaneous T-cell lymphomas and from their cell lines which are established in vitro. These viruses appear to be unique and it is believed that this is the first concrete

demonstration of an association of oncogenic viruses and human malignancies. We have also shown that several other patients have antibody against this virus. These studies are continuing at present.

2.6 Multiple Myeloma Treated with Alternating Combination Chemotherapy (NCI-VA Protocol 76-7; Dr. Bunn, Senior Staff in collaboration with Dr. Shechter, Hematology, VAMC).

2.601 Multiple Myeloma and other Plasma Cell Malignancies

Patients with multiple myeloma and Waldenstrom's macroglobulinemia are treated with alternating active chemotherapy regimens. The first regimen consists of melphalan and prednisone which are given for 15 weeks. Following this patients are given a 4 drug combination of vincristine, cyclophosphamide, adriamycin and prednisone for 14 weeks, following which patients receive a three drug combination of BCNU, melphalan and prednisone for 15 weeks. The patient's then receive again the four drug regimen of vincristine, cyclophosphamide, adriamycin and prednisone. The study was designed to test whether these alternating regimens would prevent drug resistance or delayed drug resistance thereby increasing the frequency of complete remission and prolonging remission duration and survival. Patients achieving a remission have been unmaintained after the 60 weeks of therapy. To date, 27 patients have entered this trial and it is anticipated that it will be closed for accrual during the next year. Preliminary results from this trial suggests that overall median survival will be in excess of 30 months which is as good as the survival in the best published series. However, the complete remission rate does not appear to be higher than in other trials. Patients reduction in M protein levels reached a plateau within 6 to 9 months of the institution of therapy, much the same as patients treated with a single drug combination. The trial does establish that chemotherapy can safely be discontinued for patients in remission. However, patients must be followed closely for progressive disease, which may occur with a median of 9 to 12 months.

2.602 Phase I - II Trial of Total Body Radiotherapy in patients with multiple myeloma and Waldenstrom's macroglobulinemia failing combination chemotherapy (Dr. Bunn and Senior Staff in collaboration with Dr. Lichter and staff of Radiation Oncology Branch).

A new trial of total body radiotherapy in patients failing combination chemotherapy has been instituted. In this study, patients with adequate hematologic function receive 50 rad of total body radiotherapy weekly to a maximum dose of 200 rads.

This trial was instituted on the basis of antedoctal reports showing that total body radiotherapy is effective in this disease and on the basis that total body radiotherapy can be safely given to patients, as shown in studies of malignant lymphomas. Second line chemotherapy has been most unsuccessful in patients who have previously failed combination chemotherapy. One patient has entered this trial to date, this patient received two 50 rad doses of total body radiotherapy. However, there was no response to the treatment in this patient.

2.7 Hepatocellular Carcinoma

2.701 Combination chemotherapy of hepatocellular carcinoma with adriamycin and streptozotocin (NCI-VA Protocol 76-11; Dr. Ihde and Senior Staff, in collaboration with Dr. McIntire, LID/DCRD).

Fifteen patients with unresectable hepatocellular carcinoma have been treated with a combination of adriamycin and streptozotocin, chosen because of the known activity of each agent used individually and their non-overlapping toxicities. Two patients had partial responses of 18 and 11+ months. As in our previous study with Adriamycin alone, the major predictor of survival was pre-treatment performance status. The response rate is similar to those previously reported by ourselves and others for adriamycin alone. Disease response and progression is being monitored with the aid of serum alpha-fetoprotein determinations.

2.8 Colon Carcinoma

2.801 Phase II trial and pharmacologic studies of PALA in Colon Carcinoma (NCI-VA Protocol 79-6 and 78-3; Dr. Cohen and Senior Staff in collaboration with Dr. Erlichman, Dr. Chabner, CPB).

Two patients have received PALA 1500 mg/M² daily times 5. Serum PALA levels were determined in both patients. Levels of aspartate transcarbamylase were measured in the skin before and after PALA. Cutaneous PALA toxicity was noted in 1 patient. No responses have been seen, to date. The study is ongoing.

2.9 Phase I Trials

2.901 Phase I Trial of weekly 6-hour infusions of dichloromethotrexate (DCM, NSC 29630) in hepatocellular carcinoma and other solid tumors. (NCI-VA Protocol 78-1; Dr. Ihde and Senior Staff, in collaboration with Dr. Myers, CPB, Dr. Levenson and Dr. Donehower.

Dichloromethotrexate (DCM) is a methotrexate analog with activity superior to that of the parent compound in murine L1210 leukemia. It has previously been studied on daily oral and 2 or 3 times per week IM schedules in human cancer, without evidence of results that were superior to those expected with Methotrexate. Because of significant hepatic metabolism, it should be more suitable than methotrexate for administration to patients with impaired renal function. We have begun a dose-finding trial, giving DCM in a weekly 6-hour infusion schedule. Pharmacokinetics are being done with the commercial radio-immunoassay for methotrexate, and an HPLC assay is being developed. Should results appear promising, even higher doses of DCM with citrovorum factor rescue may be studies. 11 patients have been treated thus far, with the dose presently at 800×2 , 1200×2 and then 1600 mg/m^2 IV weekly. Severe hematologic toxicity has been observed in 3 patients; only minimal mucositis and myelosuppression or no toxicity at all have been noted in the remaining patients. Three partial responses, in patients with adenocarcinoma of unknown primary site and hepatocellular carcinoma have been observed.

2.902 Phase I trial of aziridinylbenzoquinone (AZQ) (NCI-VA Protocol 80-2; Dr. Ihde and Senior Staff, in collaboration with Dr. Schilsky and Senior Staff, MG, and Dr. Poplack, POB).

AZQ is a rationally synthesized drug in which the molecular characteristics of good lipid solubility and low ionization (in order to penetrate the central nervous system) and good water solubility are optimized. It may function as an alkylating agent. This Phase I trial under the leadership of Dr. Schilsky was only recently initiated. Three NCI-VA patients have been entered thus far. Although dose-limiting toxicity has not been reached, some myelosuppression has already been observed.

2.903 Phase I Trial of Pentamethylmelamine (PMM, NSC 118742)(NCI-VA Protocol 78-5; Dr. Ihde and Senior Staff in collaboration with Dr. Young, MB, and Dr. Dutcher and Dr. Boyd, CPB).

Pentamethylmelamine (PMM) is a water-soluble analog of hexamethylmelamine (HMM) which is suitable for intravenous administration. HMM can be given only orally, and its dose-limiting toxicity is often gastrointestinal; it has activity against lung, ovarian, and other

cancers, but its absorption from the GI tract is erratic. Thus, a suitable parenteral schedule of PMM, which has equivalent therapeutic ratios to HMM in several animal tumors, should be of considerable clinical interest. A gas chromatographic/mass spectrometric assay for PMM has been developed by the CPB. Patients from the NCI-VA MOB and the MB are entered onto study.

The Phase I trial is now complete after 34 patients have been entered. Intolerable toxicity of nausea and vomiting that persisted for up to 24-48 hours and was unresponsive to antiemetics was reached at the dose of 1500mg/m². Prolongation of the infusion time to 6 hours at this dose modestly reduced the toxicity. Minimal lethargy and somnolence was the only other dose-related toxicity. Consistent myelosuppression was not observed. No complete or partial responses were observed. Pharmacokinetic studies revealed a peak level at the end of infusion that was proportional to dose and an elimination half-life of 52-370 minutes that was independent of dose. The maximum tolerated dose recommended for Phase II trial is 100 mg/m² per week. Its lack of myelosuppression at this dose would make PMM an interesting agent for further development if antitumor activity could be defined at tolerable doses.

2.10 Staging Studies and Evaluation of Tumor Markers in the Natural History and Management of Malignant Diseases.

2.1001 Fiberoptic bronchoscopy (Dr. Ihde, Dr. Cohen, and Dr. Carney)

During this year fiberoptic bronchoscopy (FOB) has been performed on an elective basis 180 times in lung cancer patients prior to or during chemotherapy. Each procedure entails thorough visual examination to the level of all segmental and many subsegmental bronchi and procurement of bronchial washings for cytologic examination and endo-bronchial biopsies. We have found that FOB is extremely useful in the management of patients with SCC. Recent review of 133 consecutive procedures in previously untreated patients about to begin combination chemotherapy revealed that the procedure was positive, either visually or pathologically, or both, in 119 patients (89%), including eight (6%) in whom FOB provided the only evaluable evidence of intrathoracic cancer. FOB is most valuable in segregating a group of SCC patients in apparent complete response to treatment by chest x-ray who retain evidence of relapse. Of 43 patients in radiographic complete response after 6 weeks of chemotherapy, 6% of 31 patients with no bronchoscopic evidence of tumor had relapsed by Week 24, while 50% of the 12 patients with positive FOB examinations despite a normal chest x-ray had relapsed by the same time point. Positive bronchoscopy and lack of complete response by chest x-ray at Week 6 were equally likely to predict chest disease progression by Week 24. In non-small cell lung cancer, pre-chemotherapy FOB examinations are less often positive for tumor (79% positive FOB's in 47 patients). Since complete responses to chemotherapy are rarely obtained in these patients, FOB is not needed to confirm total clinical absence of tumor; however, it has been found to be useful in monitoring for early progression of tumor. Of 21 episodes of chest tumor progression on chemotherapy, 38% were first detected by FOB alone (before radiographic tumor enlargement was evident). These results were recently published (*Cancer* 45: 1693, 1980). Bronchial washings, a repeatedly accessible source of tumor cells in many lung cancer patients, are being studied in several in vitro systems.

2.1002 Peritoneoscopy (Dr. Bunn, Dr. Carney, Dr. Radice, Dr. Cohen)

Peritoneoscopy with multiple liver biopsies is being investigated as a staging and restaging procedure in patients with cutaneous T-cell lymphomas and small cell lung cancer.

Small Cell Lung Cancer

Pretherapy staging peritoneoscopies have been performed in approximately 60 patients who have had negative or inadequate percutaneous liver biopsies. We have found many positive liver biopsies when patients were not suspected clinically by scan or by abnormal liver function tests. All patients with positive biopsies at presentation have had lesions which could be seen visually, which is in contrast to findings in the malignant lymphomas. In addition to being useful as a pretherapy staging procedure we have found peritoneoscopy useful in obtaining tissue for our in vitro growth and for flow cytometric studies. At present we are reviewing our experience with peritoneoscopy and preparing this material for publication.

Cutaneous T-cell Lymphomas

We have shown that percutaneous liver biopsy is inferior to peritoneoscopy with multiple liver biopsies for establishing a diagnosis of lymphoma involvement of the liver and cutaneous T-cell lymphomas. In addition, the physical examination and liver function tests are inadequate for determination of liver involvement. We have also shown that patients with generalized erythroderma, patients with leucocytosis, patients with lymphocytosis patients with lymph node involvement are more likely to have liver involvement than patients without these findings.

Other Malignancies

Peritoneoscopies are performed to document liver involvement in patients with various malignancies undergoing work up to determine whether their primary tumor is the sole site of tumor or whether they have metastatic disease. Documentation of liver metastases in these circumstances can spare patients a laparotomy.

2.1003 Computerized tomography (CT scans) of abdomen, pelvis, and in various malignancies. (Drs. Ihde and Bunn, in collaboration with Dr. Dunnick, DR/CC, and Dr. Bates, Nuclear Medicine Service, WVAMC).

In SCCL, involvement of retroperitoneal structures is often present at autopsy, but difficult to detect clinically. Similarly, although brain metastases are a frequent clinical and pathologic occurrence, screening radionuclide brain scans in asymptomatic patients have been unsuccessful in detecting earlier perhaps more easily

treatable CNS metastases. We have performed abdominal CT scans in 75 patients previously untreated SCCL patients. CT scans of liver were approximately as useful as radionuclide liver scans in predicting pathologically confirmed liver involvement. More importantly, adrenal or retroperitoneal masses were found in 11 patients (13%). A preliminary report of these findings has been published (Am. J. Roent. 133, 1085, 1979). Follow up studies during treatment to determine the utility of this technique in patient management are ongoing. Pre-treatment CT scans of the head in SCCL patients have recently been initiated, as have CT scans of the abdomen and pelvis in prostatic and gastric cancer, two tumors in which evaluation of chemotherapy responses is notoriously difficult to document. Further studies must be accumulated before the contribution of CT scans in the latter two diseases can be analyzed. In patients with hepatocellular carcinoma, abdominal CT scans have been compared to radionuclide liver scans. CT scan detected abnormalities in 6/7 patients with abnormal radionuclide scans. The two techniques were complementary in assessing response to chemotherapy, since each detected some episodes of tumor response or progression that were not initially apparent with the other modality. These results have been published (J. Comp Assist Tomogr 4:59, 1980)

2.1004 Lymphangiography in prostatic cancer and mycosis fungoides (Drs. Ihde and Bunn, in collaboration with Dr. Dunnick, DR/CC).

Detection of response to chemotherapy in prostatic cancer is often difficult. We have obtained pre-treatment lymphangiograms (LAG's) in our patients, and then periodically assessed nodal status with follow-up abdominal and pelvic x-rays. Thus far, LAG's have yielded evaluable evidence of nodal metastases in about 60% of our patients with Stage D prostatic cancer, (Cancer 45:300, 1980). Partly because of dye elution, serial abdominal films have thus far not been helpful in detecting tumor progression. In mycosis fungoides, pre-treatment LAG's have revealed positive nodes in about 70% of patients with palpable peripheral adenopathy, and have always been negative in patients without such physical findings. In contrast to other lymphomas, LAG's in mycosis fungoides tend to be more abnormal in more caudad nodes. The value of serial abdominal films in evaluating nodal response to chemotherapy is being assessed.

2.1005 Bone marrow biopsy in small cell carcinoma (Drs. Ihde, Matthews and Simms).

This data has been published (Blood 53; 677, 1979) and is no different from that in Sec. 2.95 of last year's Annual Report.

2.1006 Tumor markers in patients with lung cancer and prostatic carcinoma (Dr. Ihde, in collaboration with Dr. McIntire, LID/DCBC, Dr. Zweig, Clinical Chemistry, CC, Dr. Ruddon, Frederick CRC, and Dr. Belville, Urology Svc., Walter Reed AMC.).

Serial serum samples from lung cancer patients obtained before and during chemotherapy are being provided to Dr. McIntire, who is developing an assay for an apparently specific lung cancer-associated antigen. The sensitivity of this assay in detecting residual disease after therapy would be a question of obvious interest. Bronchial washings from tumor-involved and contralateral lung are being assayed by Dr. Ruddon for a panel of tumor-associated marker substances. Results are being compared to plasma samples from the same patients on whom bronchoscopic material was collected. Dr. Belville has completed determinations of prostatic acid phosphatase by a radio-immunoassay technique on serial serum samples from patients with carcinoma of the prostate undergoing chemotherapy. The comparison of serial determinations in enzymatic and RIA acid phosphatase values in thoroughly staged patients has unfortunately revealed that RIA provided little additional information in comparison to enzymatic methods. The utility of the serum levels of the BB enzyme of creatinine kinase in assessing response to therapy in prostatic cancer is being studied with Dr. Zweig. In addition, we have found that the BB isozyme is a marker of SCC with levels over 100 times higher than in non-SCC. We are retrospectively and prospectively studying our serum bank for this marker.

2.1007 Influence of sites of metastases on prognosis in patients with SCC (Dr. Ihde, in collaboration with Dr. Makuch, BRB).

The influence of various sites of metastatic disease on complete response rate and survival is being analyzed in 106 consecutive completely staged previously untreated patients with SCC. All patients received intensive induction chemotherapy. Minimum follow-up time is 2 years. Preliminary results indicated that: 1) performance status is the dominant prognostic factor; 2) the number of sites of metastatic disease correlates quite well with performance status; 3) patients with a single site of distant metastatic disease fare no worse than those with limited disease; 4) prognosis is inversely related to the number of sites of metastatic disease; and 5) liver and central nervous system are the only initial sites of metastatic involvement that adversely affect survival, when other factors are statistically accounted for.

2.1008 Development of Metastases in Sanctuary Sites in Small Cell Lung Cancer and T-cell Lymphomas (Dr. Bunn and Senior Staff in collaboration with Dr. Dunnick, Department of Radiology, Clinical Center and Dr. Glatstein and Senior Staff, Radiation Oncology Branch

Small Cell Lung Cancer: We have shown that central nervous system metastases are extremely common in patients with small cell lung cancer being present at diagnosis in 10 per cent of patients and progressively increasing with time.

These metastases are present in over 80 per cent of patients at risk for two or more years. In addition we have shown that leptomeningeal and spinal metastases are very frequent in these patients and their frequency also increases with time. We have shown that radionuclide brain scans and lumbar punctures are not useful for detecting lesions in asymptomatic patients. At the present time CAT scans are being evaluated for their ability to detect central nervous system metastases in asymptomatic patients. To date, at least 4 patients have had such asymptomatic lesions detected by CAT scans. This study is in progress.

We have shown that combination chemotherapy is not effective in reducing the frequency of CNS metastases including chemotherapeutic agents which cross the blood brain barrier. Therefore, in conjunction with the Radiation Oncology Branch we have instituted a trial of prophylactic cranial irradiation. This study is currently in progress and results are being evaluated at present.

Cutaneous T-Cell Lymphomas.

We have shown that skeletal metastases are not rare in patients with cutaneous T-cell lymphomas. These skeletal manifestations may include osteoblastic lesions, osteolytic lesions or generalized osteoporosis. We have shown, in collaboration with Drs. Schechter and Horton, that a sizeable protein factor termed osteoclast activating factor "OAF" may be responsible for causing osteolytic lesions and generalized osteoporosis. We have also shown that pulmonary metastases are relatively frequent in these disorders, although they are uncommon at diagnosis their frequency progresses with time. We have also shown that these lesions may be infiltrative lesions or may be manifested clinically as pulmonary nodules. We have also shown that lymphomatous leptomeningitis can develop in patients with cutaneous T-cell lymphomas. A review of the clinical features of cutaneous T-cell lymphomas has been published by Drs. Carney and Bunn.

2.1009 Analysis of Long Term Disease Free Survivors of SCCL Who are Potentially Cured. (Dr. Minna and Senior Staff in collaboration with Dr. Glatstein and the ROB).

The NCI trials in SCCL have had as their goals not only to increase the quality and duration of life for the majority of patients entered, but also to attempt to cure a fraction of these patients. We have reviewed 168 patients accessioned into NCI protocols from March, 1973 until July, 1977 to allow for at least 35 months or more of follow-up. We have arbitrarily designated for this analysis as long term survivors, all patients that were alive and without clinical evidence of SCCL 30 months or more from the start of treatment. Twenty patients of the 168 or 12% overall met this requirement.

The patients had been treated on approved protocols at the NCI-VA Branch, and the Radiation Oncology Branch. All protocols involved the use of initial intense combination chemotherapy with or without chest radiotherapy. 41% of the patients had limited stage disease and 59% had extensive stage disease after multiple pretreatment staging procedures. Six to 12 weeks after the start of therapy, at the completion of the initial intensive induction period, the patients were restaged and classified as having either no clinically evident tumor, a complete response, or lesser tumor responses scored as partial or no responses. 53% of all patients treated entered a complete response.

Of the 168 patients 25% of all limited stage patients and 3% of all extensive stage patients were in the long term disease free survivor category, a significant difference. The median survival of this group of 20 patients is now 45+ months with a range of 36+ to 75+ months from the start of the treatment. Analysis of this group revealed that all had entered an initial complete response and had remained in it until the 30 month time point.

Another factor contributing to the good result besides limited stage disease and an early complete response were the initial good performance status with 85% of the group being fully ambulatory. Also, retrospective analysis revealed that 19% of the long term survivors received chemotherapy plus radiotherapy while only 5% of them received chemotherapy alone, a significant difference which persisted after being adjusted for disease stage. While the patients in some protocols had been projected to receive maintenance chemotherapy for 2 years, the median length of all chemotherapy was 4 months with a range of 2 - 22 months. The median time off of chemotherapy was 36 months with a range of 18-60 months.

The current status of the group of 20 long term survivors is as follows: 17 are still alive, and only one patient in the 20 has relapsed from SCCL (this at 53 months from the start of treatment). 10 are asymptomatic; and 6 have varying degrees of symptomatology related either to their treatment or underlying chronic pulmonary disease. Seven patients who received radiotherapy have varying degrees of pulmonary fibrosis. The three patients that have died have all done so without evidence of SCCL. However, one died of erythroleukemia at month 40 which is felt to be a complication of therapy.

We concluded from the analysis of the group of long term survivors from the NCI trials that: intensive initial therapy can yield up to 12% in this category; that these patients initially enter and remain in complete response while no partial responder has lived past 30 months in the NCI trials; the majority of these survivors have initial excellent performance status; patients with limited stage disease yield significantly more long term survivors; that only a short period of 4-6 months of chemotherapy may be needed; and that retrospectively, combined modality chemotherapy and radiotherapy yielded more survivors than chemotherapy alone. Future trials need to be directed at: the role of combined modality therapy; to increasing the initial complete remission rate and the reduction in both early and late treatment related complications so as to increase the number of long term, disease free survivors with good quality of life.

2.11 Clinical Pathologic Correlation in Patients with Malignant Diseases (Drs. Matthews and Gazdar and Senior Staff).

2.1101 Correlation of Clinical Response with Subtype of Small Cell Carcinoma of the Lung (SCCL).

In previous studies, it has been shown that there is no difference in behavior or response to therapy of patients with classic small cell tumors, classified either as lymphocyte-like (WPL #21), or intermediate (WPL #22). A subset of small cell tumors are characterized by clusters or nests of cells with nuclear and cytoplasmic characteristics of large cell carcinoma. This subset has been designated as mixed (WPL #22/40). There is no difference in patient characteristics, performance status, extent of disease at presentation or biologic behavior, as identified by staging procedures prior to therapy or at autopsy. When subjected to intensive high dose chemotherapy protocols, patients with #22/40 subtype had an objective response rate of 56%, in contrast with patients with subtype #21 and #22 who had response rates of 90%. Patients with subtype #22/40 also had a decreased median survival (6.6 months as compared to 10.2 months), a shorter time to relapse in responders and a particularly impressive decreased survival in patients presenting with limited disease and good initial performance status who otherwise would be good candidates. In spite of this, 1 patient with subtype #22/40 has survived over 2 years.

2.1102 Intra and inter observer variability in the histopathologic classification subtypes of small cell carcinoma of the lung (Dr. Matthews, Dr. Fred Hirsch, Finsen Institute, Copenhagen, Denmark and Dr. Raymond Yesner, VAMC, New Haven, Connecticut.)

This study was made to 1) evaluate and compare the reproducibility of the 1967 and 1977 WHO Lung Cancer Classifications of small cell carcinoma of the lung (SCCL); 2) identify microscopic characteristics possibly useful in distinguishing subtypes; and 3) identify problems which persist in the diagnosis of this tumor. One of the participants, (FH) initially studied and classified slides of 93 patients with SCCL, according to both WHO classifications. The slides were blindly classified by the other 2 authors.

The distribution of cell type by the initial reviewer using the 1967 criteria was lymphocyte/oat cell, 38%; fusiform 32%; polygonal cell, 37% and others, 6%; with the 1977 criteria, lymphocyte/oat cell, 58%; intermediate, 37% and combined 4%. Agreement in the diagnosis of small cell carcinoma was 94% (1967) and 97% (1977). Unanimity in subtyping was present in 38% (1967) and 54% (1977). Unanimity in the subtyping of the lymphocyte/oat cell group was 69% (1967) versus 85% (1977). Tumors classified as

intermediate had a statistically significant higher incidence of fibrous stroma and inflammatory host responses than the other subtypes. In 16 instances, slides were interpreted by at least one reviewer as SCCL with mixed large cell component. In only 4 of these cases did a second reviewer agree with the diagnosis. Four of the 16 cases would not have been placed on a small cell protocol by at least one reviewer. It is concluded that a high degree of consistency in the diagnosis of SCCL is possible; however, inconsistencies in subtyping are common. The 1977 WHO classification tends to increase consistency in subtyping, particularly of the lymphocyte/oat cell group. Additional criteria and agreement are required to identify small cell tumors admixed with other histologic types.

2.1103 Consistency in the Diagnosis of Small Cell Carcinoma Versus Non-Small Cell Carcinoma of the Lung. (Drs. Matthews and Gazdar, Dr. Michael Jones, Albert Einstein School of Medicine, Dr. Thomas Bonfiglia, University of Rochester, New York, Dr. Joseph Eggelston, Johns Hopkins University, Dr. Steven Goldberg, Mt. Sinai Hospital, New York, Dr. Fred Hirsch, Finsen Institute, Copenhagen, Denmark, and Dr. Louis Ortega, VAMC, D.C.).

This study was undertaken to evaluate the degree of consistency in, and reproducibility of, the diagnosis of small cell carcinoma and to identify problem areas. For this purpose eight pulmonary pathologists involved in cooperative studies, reviewed one-hundred coded histological (H & E stained) or cytological preparations from broncho-pulmonary tumors.

Seventy-five of the cases were known small cell tumors presenting diagnostic problems. Twenty-five of the tumors were non-small cell tumors (poorly differentiated epidermoid or adenocarcinoma, large cell carcinoma and/or carcinoids). In 23 cases (including small cell and non-small cell tumors) duplicate sections or different samples from the same tumor were used to test intra-observer consistency. In 72% of the cases there was unanimous agreement on typing of tumors. In the remaining 28% there were a total of 42 discrepant diagnoses. In small cell tumors, the most common discrepancy was a diagnosis of large cell carcinoma, followed by poorly differentiated epidermoid and adeocarcinoma. Stratification, necrosis with pseudo-glandular or papillary formation and loss of small cell nuclear characteristics due to poor fixation were the most common reasons for these discrepant diagnoses.

In the non-small cell tumors, including carcinoids a diagnosis of small cell carcinoma was made in 12 instances. This

predominantly involved incorrect interpretation of carcinoids. Five of the 8 original participants have recently re-reviewed cases which caused discrepant diagnoses. This material is being studied.

2.1104 Development of Different Lung Cancer Histologic Types after Treatment with Chemotherapy and/or Chemotherapy and Radiotherapy. (Drs. Matthews and Gazdar).

It was noted in a study from this Branch that in one-third of SCCL patients subjected to either chemotherapy or chemotherapy plus radiotherapy, there were changes in the histologic typing of the tumor. These changes included focal maturation of the small cell tumor to squamous nests, glandular elements, anaplastic large cells, syncytial giant cell formation. Tubule formation was identified in another. Some residual evidence of small cell tumor was present in all patients autopsied. This transformation post therapy to other histologic cell types implies that the progenitor cell of this neoplasm is entodermal rather than neuroectodermal in origin. Thus, all lung cancer types may have a common stem cell. A similar transformation of small cells occurs following tissue culture or nude mice heterotransplants maintained for over two years. This is associated with loss of biologic and biochemical APUD markers. It is possible this phenomenon. It is possible that this phenomenon is responsible for the failure of some patients to respond to further therapy following relapse.

2.1105 Small Cell Carcinoma Registry Long Term SCCL Survivors. (Drs. Matthews and Minna, in collaboration with Drs. Rozencweig and Muggia).

A registry of patients with SCCL who have survived over 2.5 years from diagnosis was established in 1977. To date, 196 patients have been submitted from cancer treatment centers in the U.S.A. and abroad. As of last evaluation, 45% of the patients received surgery as a primary modality, with or without chemotherapy/or radiotherapy, 45% received radiotherapy alone or with chemotherapy; 8 received chemotherapy alone. One patient had no treatment. Long term survival was achieved in all histologic subtypes including the mixed group (WPL #22/40). Over 30% survived over 5 years. Relapse recurred rarely after 3.5 years. An increasing number of patients with hepatic metastases at presentation are being included in this registry and indicate these supposedly poor risk patients may also have long term survival.

2.1106 Correlation of Histologic Subtype with Survival in Surgically Resected Lung Cancer. (Dr. Matthews in collaboration with Drs. George Higgins and Thomas Shields, VAMC, and Mr. Robert Keehn, Statistician, National Research Council).

The pathologic data and representative sections of over 800 patients with carcinoma of the lung resected by the VA Surgical Oncology Group have been reviewed. There has been a consistent discrepancy of 20% in diagnoses between the primary and reference pathologist. To date, 567 patients have been evaluated to determine the influence of cell type on long term survival. 52% of the patients have had squamous cell tumors, 30% adenocarcinoma, 13% large cell carcinoma, 3% small cell carcinoma, and 2% others.

In patients with T₁ No Mo disease, the four year survival was greater for squamous cell than adenocarcinoma (63.5 or 54.9) but not significantly so. In patients with T₂NOMO, results were similar (53% versus 46.5%). When lymph nodes were involved in either T₁ or T₂ tumors, a greater difference in 2 year survival was noted for squamous tumors than those with adenocarcinoma (56% and 36.7% respectively). The small number of large cell carcinomas does not permit long term evaluation, but they appear to have a survival similar to patients with adenocarcinomas. In a related study, however, if all epidermoid carcinomas are compared to all large cell carcinomas, regardless of tumor size or lymph node metastases, 61.7% of patients with epidermoid carcinoma survived 3 years, whereas only 36.3% of patients with large cell carcinoma survived 2.5 years. In contrast, 50% of patients with adenocarcinoma survived 2.5 years. It is thought that there may be some prognostic value in segregating large cell carcinomas from other cell types. To date, approximately 30 cases of small cell carcinoma have been resected in this surgical protocol. Information concerning survival and response to therapy is to be obtained.

2.1107 Histologic Criteria for Skin and Lymph Nodes in Cutaneous T-Cell Lymphomas (CTCL). (Drs. Matthews and Gazdar, in collaboration with Dr. Fischmann, Dermatology, Dr. Schechter, Hematology, Drs. Guccion, Patterson and Ortega, Laboratory Service WVAMC, DC.).

A blinded study was devised to develop a working histologic criteria used for the diagnosis of CTCL (Mycosis fungoides/Sézary syndrome-MF/SS). One hundred and four sections of skin biopsies were reviewed by three pathologists and one dermatologist. These biopsies included pre and post therapy skin sections from different sites on 26 patients with documented MF/SS. Diagnostic slides were not necessarily included for all patients. Sequential slides were also received on 3 patients with skin disease clinically simulating MF, but

on whom a diagnosis of MF had not been established. In addition, the study included slides from 24 patients with chronic lichenoid or psoriaform dermatitis. A form asking 45 variable questions was developed and filled out by all participants.

In 18 of the 25 patients with MF, three pathologists unanimously felt that one, or frequently 2 skin biopsies were diagnostic of or compatible with MF. Similar unanimous opinions of MF were made on 2/3 patients thought to have disease simulating MF clinically. These patients are being staged and followed at present. In all but one of the remaining 7 patients with mycosis fungoides, 3/3 or 2/3 pathologist considered that sections were were diagnostic, compatible, or that mycosis should be ruled out. One had an essentially nondiagnostic section. Mycosis fungoides was not diagnosed on any of the chronic psoriaform or lichenoid dermatites. However, frequent suggestions were made that MF should be ruled out. The forms are in the process of being evaluated to summarize significant features used in the diagnosis of this disease.

Lymph nodes removed surgically from 53 patient with CTCL have been reviewed. Cytospin surface markers, electron microscopic, and cytogenetic studies have been performed on the majority of these lymph nodes. Almost all nodes except for those frankly effaced by tumor showed dematopathic lymphadenitis in paracortical zones. A classification devised to quantitate the numbers of convoluted T-cells or mycosis cells in these paracortical regions is being evaluated for possible prognostic value. In sequential lymph node biopsies from selected patients, the number of convoluted cells increased, and the dermatopathic histologic pattern became less pronounced.

2.1108 Lymphomatoid Granulomatosis (LYG) A search for its Nature (Drs. Matthews and Meera Kanhouwa, in collaboration with Dr. Michael Janis, Albert Einstein College of Medicine, New York).

The cellular infiltrate of LYG is composed of immature lymphocytes and atypical histiocytes which have a marked tendency to invade and obliterate blood vessels producing mass lesions which cavitate and may disappear spontaneously. Such cellular components and behavior is reminiscent of CTCL. The disease has been considered a primary pulmonary entity. Although up to 20% terminate as some atypical form of lymphoma. LYG has been excluded from the lymphomas because of the relative lack of significant reticuloendothelial involvement and the lack of effacement of nodal architecture by atypical cell until late in the course of the disease. In a review of the literature, 243 cases were found. From these 49 cases, including 3 from NCI-VA sufficiently adequate information was obtained to develop a profile of

sites of organ involvement at presentation, therapeutic modalities, interval and time of recurrence or progression and interval from onset of symptoms to death. 5 additional cases from Albert Einstein College of Medicine are to be included. Of the 49 cases evaluated to date, the median age was 44; with a 2:1 male to female distribution. 71% of the patients were dead at time of report. All but one of these had disease clinically at the time of death or at autopsy. The median time to death from onset of symptoms was 1 year. The median survival of the remaining 29% who were alive at the time of report was 2.5 years. The most common sites of involvement included lung (90%) skin 61%, cranial and peripheral nerves 33%, CNS and lymph nodes 27%. Other organs identified as sites of disease included the liver, upper respiratory tract, spleen, kidneys, adrenals, retroperitoneal soft tissues and colon. CNS lesions were usually multiple, involving the cerebrum, pons, brain stem cerebellum, leptomeninges and in one instance the cord.

In 10 of the 49 cases, some form of lymphoma often termed "atypical" was diagnosed at some time during the course of the disease or at autopsy. CSF in 4 patients with CNS disease was interpreted as malignant in 3 and "atypical" in one. A variety of single agent chemotherapy agents have been used in combination with prednisone.

Radiotherapy and intrathecal methotrexate were used predominantly on patients whose tissues had been diagnosed as malignant. 8/49 patients received radiation therapy during the course of this illness. 2/8 were alive with stable disease. The median survival of the 8 cases was 6 years.

LYG is probably a malignant disease. A multidisciplinary interest in this entity could establish or deny this fact. Study of cell membrane markers, chromosomes and electron microscopy data are needed. Should this prove to be a malignant disease, appropriate radiotherapy/chemotherapy protocols should be devised.

2.1109 Reference and Consultant Pathologist Tasks:

The NCI-VA MOB pathologist, Dr. Matthews, has served as a reference or consultant pathologist for the following NCI related oncology activities. These activities have been done without clerical, technical or monetary reimbursement to this unit.

1. NCI Small Cell Carcinoma Registry (1977- present).

The majority of these materials have also been reviewed by Dr. Raymond Yesner. A panel of pulmonary pathologists has also reviewed cases presenting diagnostic problems.

2. VA Surgical Oncology Lung Group (Drs. Higgins and Shields, principal investigators) (1973-present). (800 patients' material reviewed).

3. Eastern Cooperative Oncology Group (ECOG) and Radiation Therapy Oncology Group (RTOG) small cell carcinoma protocols (1975-present). Approximately 450 forms and slides have been reviewed to date on these patients.

4. NCI-Lung Cancer Study Group (LCSG) (1977- present).

The NCI-VA pathologist has acted as a consultant to this group since its inception in 1977. The group replaced the former Working Party for Therapy of Lung Cancer and admitted new universities to the group, including Toronto, Seattle, UCLA, and Vanderbilt. Members of the pathology panel are requesting that Dr. Matthews be made a member of the group, rather than a consultant, so that she could participate in the routine review of slides. At present, all discrepancies in diagnoses are brought to biannual meetings for discussion and interpretation.

5. VA Surgical Oncology Group, Colon (Dr. Higgins, Chairman), 1979-

The NCI-VA pathologist has been requested to be the reference pathologist for the VASOG colon. Materials will be collected by the statistician, Mr. Robert Keehn (National Research Council) and will be reviewed only when pertinent questions are asked.

6. GI Tumor Oncology Group, Pancreatic Protocol, (1978-present)

Dr. Matthews is a reference pathology for the pancreatic cancer protocol of Georgetown University Hospital. This protocol is a part of the GITOG. To date, no sections have been received.

7. NCI - Small Cell Carcinoma - Hyperalimentation Protocol.
Dr. William DeWys, NCI-DCT, Project Director.

Dr. Matthews will serve as reference pathologist for this multi-institutional program, which anticipates admitting between 200 - 300 patients to the protocol. Clerical and related tasks will be accomplished by the groups' data management personnel. (Information Management System (IMS), located in Bethesda, Maryland.

2.12 Infectious Disease Studies in Patients with Malignant Disease (Dr. Fossieck and Senior Staff).

2.1201 Staphylococcal Septicemia in Cancer Patients (Dr. Fossieck, Dr. Carney, and Dr. Parker, ID, WVAMC).

45 cases of septicemia due to Staphylococcus aureus have occurred in adult cancer patients and counted for 23% of the bacteremias on the oncology unit. Twenty-five episodes occurred in patients with hematologic malignancies (23 in patients with cutaneous T-cell lymphomas CTCL) and 20 in patients with solid tumors. Predisposing factors included progressive neoplastic disease (20 cases) and a performance status of 3-4 (26 episodes). A primary source for entry of the bacteria into the blood was identified in 34 episodes and included active CTCL skin lesions (20), intravenous sites (5), mucositis (5), and miscellaneous sites (4). Severe granulocytopenia (<500) was present present in only 6 cases. Clinical signs and symptoms were similar to those found in sepsis due to other microorganisms. The patients survived in 38/45 (84%) cases. Complications were uncommon and included 3 gram bacillary superinfections during therapy, pneumonia in 3 cases and endocarditis in 1.

2.1202 Staphylococcal Sepsis in Cancer Patients: Intravenous and Oral Antimicrobial Therapy (Dr. Fossieck, Dr. Carney, and Dr. Parker, I.D., WVAMC).

Eighteen adult cancer patients with 21 episodes of staphylococcal bacteremia were treated with sequential intravenous and oral antimicrobials. Adequacy of antimicrobial therapy was monitored with serum antibacterial activity studies. The mean duration of intravenous and oral therapy was 9 and 25 days respectively. Clinical and bacteriological cures were achieved in all cases except 1 that relapsed after only 18 days of therapy. One patient had staphylococcal endocarditis and 1 had staphylococcal pneumonia. Four patients died of causes unrelated to staphylococcal bacteremia after 12, 21, 27, and 40 days of therapy. Initial intravenous therapy followed by oral antimicrobials to complete treatment, monitored with serum antibacterial activity studies, is effective therapy for patients with cancer and staphylococcal bacteremia and can avoid the morbidity, limitation of activity, and extended hospitalization associated with prolonged intravenous drug administration.

2.1203 Septicemic Complications of the Cutaneous T-Cell Lymphomas. (Dr. Fossieck, Dr. Posner, and Dr. Bunn).

The records of 60 consecutive patients with cutaneous T-cell lymphomas were reviewed to determine the incidence, etiology, pre-disposing factors, therapy, complications and outcome of septicemia. Fourteen (23%) patients had 26 septicemias: 21 due to Gram positive cocci and 5 due to Gram negative bacilli. Stage IV lymphomatous disease ($p=0.014$), and histologic involvement of lymph nodes ($p=0.023$) and peripheral blood ($p<0.001$) identified a subset of patients at high risk for sepsis. Sepsis was correlated with locally infected sites in 77% of episodes. Single antimicrobial therapy was successful for all septicemias due to Gram positive cocci, but was accompanied by 5 secondary Gram negative bacillary superinfections (80% fatal). Therefore, patients with extensive lymphomatous skin lesions, colonized with Gram negative bacilli, should receive combination antimicrobial therapy for Gram positive coccal septicemia. The subsequent mortality in all patients surviving infection (50%) indicated their poor overall prognosis.

2.1204 Counterimmunoelectrophoresis of Staphylococcal Teichoic Acid Antibody in Cancer Patients. (Dr. Fossieck, Dr. Carney, Mr. Fedorko Microbiologist.

Counterimmunoelectrophoresis (CIE) for the detection of staphylococcal teichoic acid antibodies (TAA) was performed at hospital admission on serum from 216 cancer patients without staphylococcal infection, on 25 following S. aureus bacteremia, and on 45 control subjects. Detectable TAA was present in 24% to 59% of uninfected patients with various types of cancer and 64% of control subjects. Of the 25 patients with bacteremia, 44% did not develop detectable TAA following S. aureus bacteremia. Titers of TAA determined by CIE in the other 14 bacteremic patients were not diagnostically helpful. On the basis of current information, the presence or absence of TAA detected by CIE should not be used to establish or exclude the diagnosis of serious S. aureus infection in cancer patients.

2.1205 Sequential Parenteral Then Peroral Antimicrobial Therapy of Staphylococcal Endocarditis. (Dr. Fossieck and Dr. Parker, ID, WVAMC)

Thirty-five episodes of staphylococcal endocarditis in 33 patients were treated with sequential parenteral then peroral, antimicrobial therapy. All patients had three or more blood cultures positive for S. aureus and all had cardiac murmurs characteristic of valvular insufficiency. The mean total duration of antimicrobial therapy was 42.4 days, consisting of a mean of 16.4 days of parenteral

therapy followed by a mean of 26 days of oral therapy. Parenteral antimicrobials included sodium nafcillin (32 cases, mean dose 9.1 g daily) and clindamycin (three cases). Oral therapy included dicloxacillin or oxacillin (30 cases, mean dose 4.5 g daily), clindamycin (four cases), and potassium penicillin V (one case). Serum bactericidal titers using the blood culture isolates demonstrated comparable activity with parenteral and oral drugs. All patients treated with this sequential parenteral and oral regimen were cured. A regimen of initial parenteral followed by peroral antimicrobial therapy, monitored with serum antibacterial activity studies, is a safe, effective, well tolerated and economical treatment of Staphylococcal endocarditis.

2.1206 Monitoring of Infection during Episodes of Severe Pancytopenia in Patients with Solid Tumor Undergoing Intensive Combination Chemotherapy and Combined Modality Therapy (Dr. Fossieck and Dr. Earle)

Potentially fatal anorectal infections are known to occur in 10% to 30% of patients treated for acute leukemia. Prior information suggests that this complication occurs in < 0.5% of all patients with solid tumors. Review of the records of 190 consecutive small cell lung cancer patients treated with intensive protocol induction chemotherapy reveals an incidence of 6% in this population with an attendant mortality of 9%. Major risk factors include a prior history of anorectal pathology and granulocytopenia of < 500 ml. Early institution of appropriate antimicrobial therapy and aggressive local therapy are required to control this problem.

2.1207 Prospective Study of Hepatitis Virus Transmission in an Oncology Unit. (Dr. Fossieck in collaboration with Drs. Seef and Hoofnagle, GI-Hepatology, WVAMC and NIH.)

This perspective study of hepatitis virus transmission was begun on our unit 2 years ago. At that time all consenting personnel were screened for the presence of hepatitis antibody and antigen. No personnel was found to harbor the antigen while 16% had antibodies. A repeat of this study 1 year ago revealed no antigen carriers and a similar incidence of antibody positive personnel. The risk of personnel acquiring viral hepatitis on the unit remains nil.

2.13 Complications of treatment, acute and chronic. (Dr. Fossieck, Dr. Cohen, Dr. Minna and Senior Staff).

2.1301 Interstitial pulmonary disease. Pulmonary toxicity of antineoplastic therapy: collaborative clinical and preclinical studies. (Dr. Cohen and Senior Staff).

Pulmonary toxicity, secondary to chemotherapy dose or to chemotherapy plus radiation therapy is heralded by acutely developing diffuse bilateral interstitial infiltrates on chest x-ray. The clinical picture is characterized by progressive dyspnea and hypoxemia and mortality is high. The pathogenesis and therapy for this complication are unknown. To further study the treatment related toxicity, a group of interested investigators, including members of the NCI-VA Medical Oncology, ROB, and Clinical Pharmacology Branches have met to design experimental approaches to evaluate this problem. In addition, a workshop on lung injury under the auspices of the Radiotherapy Development Branch was also held on April 18, 1980. Collaborative studies will be initiated during the coming year.

2.1302 Development of second malignancies in long term survivors of small cell lung cancer and other malignancies. (Dr. Bradley, Dr. Minna and Senior Staff, in collaboration with Dr. J. Wheng-Peng, MB, NCI).

We are monitoring our long term SCCL survivors for the development of pancytopenia and acute leukemia. Hematologic abnormalities have developed in 3 of 8 such patients, and acute leukemia in 1. Prospective cytogenetic studies to identify such patients are underway.

2.14 Rehabilitation and nutritional support of cancer patients.

2.1401 Enteral nutrition during intensive induction combination chemotherapy and combined modality therapy. (Dr. Cohen and Senior Staff).

A major problem facing intensive chemotherapy of solid tumor is nutritional support of patients. In protocol 77-2, the patients undergoing 6 induction cycle lost 7% of their dry body weight, and caloric intake was grossly diminished. This was in some cases, but not the majority, secondary to mucositis. These other factors remain to be identified. While total parental nutrition can be undertaken in these patients it involves substantial expense and patient care time. Another approach is the use of enteral nutrition via small feeding tubes. We have piloted several patients during induction chemotherapy with enteral feeding. This was well tolerated and weight stabilization and gain achieved.

2.1402 In addition, we are beginning evaluation of intense oral mucous membrane care in collaboration with the Dental Service (Dr. Redman) WVAMC, during intensive combination chemotherapy as an aid to increase nutritional support of patients.

2.1403 Study of the effect of smoking abstinence on survival of treated patients with small cell lung cancer (Mrs. Johnston-Early, Dr. Cohen and Senior Staff in collaboration with Dr. Makuch, Biometry Branch, NCI).

To determine the prognostic effect of permanent cessation of cigarette smoking, either at the time of diagnosis or prior to diagnosis of small cell lung cancer, 112 newly diagnosed patients were studied. Twenty individuals had permanently stopped smoking prior to diagnosis (NS-Prior), 35 had stopped at diagnosis (NS-Dx) and 57 patients continued smoking (S). Therapies included chemotherapy with or without radiation therapy and/or Thymosin fraction V. The survival difference among the 3 groups was significant ($p=.035$). NS-Prior patients had the best survival experience, following by NS-Dx patients and finally S patients. No S patient has survived, disease-free, more than 96 weeks, while 3 NS-Prior and 3 NS-Dx patients are disease-free 103-220 weeks after start of treatment. Thymosin (60 mg/m^2) yielded survival benefits for the S group only ($p=.022$). Continuation of smoking during the treatment of small cell lung cancer appears to worsen prognosis, while discontinuation of smoking, even at diagnosis, may have beneficial survival effects.

2.15 NURSING SECTION

2.1501 Eight abstracts were submitted to the Fifth Annual Congress of the Oncology Nursing Society, May 27-30.

One abstract was accepted for presentation.

Two audiovisuals were accepted for presentation.

One nurse submitted an abstract which was accepted for presentation at the ASCO meeting.

Some nurses on the Unit have been active in the local Cancer Nurses Association and presented a case study at the January meeting.

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PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Laboratory Investigation of Tumor Cell Biology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

See attached sheet

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NCI-VA Medical Oncology Branch

SECTION

Human Tumor Cell Biology Laboratory

INSTITUTE AND LOCATION

Washington VAMC Washington, D.C. 20422

TOTAL MANYEARS:

17.5

PROFESSIONAL:

8

OTHER:

9.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☒ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project uses a multidisciplinary approach to study tumor cell biology so as to understand the basic nature of human malignancy and to develop methods for the diagnosis and control of human cancer. Particular emphasis is placed on lung cancer and cutaneous T-cell lymphomas. Our major efforts are in the growth of human tumors in vitro and in the nude mouse to study the differentiation, cell kinetics, immunology, experimental therapy, biochemistry, growth factor requirements, tumor markers, and ectopic hormone secretion in these model systems. The human tumor colony forming and nude mouse xenograft assays are used to study tumor biology and to test tumor sensitivity in vitro. Another major area is the use of somatic cell hybrids to study tumor cell biology and cell genetics. These include production of monoclonal antibodies by hybridomas against tumor antigens and defined proteins, comparative gene mapping, human hormone production, and genes controlling expression of the malignant phenotype. Other areas studied include tumor blood flow, tumor cell kinetics, flow cytometric analysis of human tumors, and DNA content of tumor samples.

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3.1 Establishment of cell culture lines from lung cancer. (Drs. Gazdar and Carney, E. Russell, Chemist, H. Sims, Biologist).

We have attempted to develop new model systems to study the biopsy and control of bronchogenic carcinomas. Long term cultures offer a continuous source of tumor cells to study expression of differentiated functions, tumor induction and behavior studies after heterotransplantation into athymic nude mice, a potential source of hormones and other growth factors that they may release, a course of antigens and immunogens to develop and test tumor specific monoclonal antibodies, a method to test growth factor requirements, and offer models for in vitro testing of new treatment modalities. We have established over 18 continuous cultures from SCBC tumors either directly from the patient, or after heterotransplantation in athymic mice. With the use of conditioned medium from other growing SCBC cultures, we have increased the success rate from less than 30% to about 75%. The cultures grow as floating aggregates, with relatively long doubling times, and clone in soft agarose at relatively low efficiencies. Cytologically, the cultures initially resemble SCBC, but with time they may alter morphologically to large cell carcinoma, as described below. They have a wide range of DNA content, ranging from near diploid to subtetraploid. All cultures are tumorigenic. Non SCBC lung cancers, epidermoid, adenocarcinoma, and large cell, have also been established in long term culture, and their biology and biochemistry are compared to SCBC cultures. We have established 6 such cultures, and obtained one other from another source. The non-SCBC lung cancer cultures grow rapidly as adherent lines, clone readily and have short doubling times. Thus, their morphology and growth characteristics contrast with those of SCBC cultures. B lymphocyte cultures have also been initiated from some patients whose tumors have been cultured. The lymphocyte cultures either arose spontaneously, or their peripheral blood lymphocytes were transformed with EBV virus. They provide useful controls for testing of monoclonal antibodies, as well as for other studies. Cultures of lung cancer are suitable models for many in vitro studies. Specifically, we wish to use them to screen for new therapeutic agents in vitro in comparison studies to the human colony forming assay. For these reasons, we plan to continue to establish and characterize such cultures.

3.101 Characterization of Small Cell Bronchogenic Carcinoma (SCBC) cultures (Drs. Gazdar, Carney, Alvarez, Radice and Minna in collaboration with Dr. Baylin, Johns Hopkins Hospital, Dr. Guccion, Laboratory Service, WVAMC, and Dr. Whang-Peng, C MO).

As described above, multiple cultures have been established from SCBC and non-SCBC lung cancers. The SCBC cultures have characteristics of amine precursor uptake and decarboxylation (APUD) cells, while non-SCBC cultures lack these features. SCBC

cultures have very high levels of the key APUD enzyme L-dopa decarboxylase, approximately 1000 fold higher than non-SCBC cultures. In addition, SCBC cultures contain easily identifiable neurosecretory granules, and fluoresce brightly after exposure to formaldehyde vapor, provided they have been pre-incubated with amine precursors. The latter finding indicates precursor uptake and decarboxylation. Over 75% of SCBC cultures elaborate one or more polypeptide hormones. We have established over 40 clones from SCCL cultures, and are characterizing them for heterogeneity of hormone secretion and expression of APUD cell markers. Preliminary cytogenetic analysis of SCBC cultures indicates that while each culture has its unique chromosome markers, a common marker may be present in all cultures. We will characterize the clones and complete cytogenetic studies, in addition to identifying other differences between SCBC and non-SCBC cultures.

3.102 Morphological and biological changes in long term SBC cultures. (Drs. Gazdar, Carney and Bunn, E. Russel, Chemist, H. Sims, Biologist, in collaboration with G. Guccion, WVAMC, S. Baylin, Johns Hopkins Hospital).

About 6% of SCBC have mixed histology at presentation, and over 37% at autopsy have other histological forms of lung cancer or have completely altered their morphology. We investigated whether similar changes occurred in long term cultures and heterotransplanted tumors. Initially, the morphology and cytology of the tumor cells were classical SCBC and maintained this for periods of up to 24 months. However, then the majority of cultures grown in vitro or in nude mice for longer than 24 months showed partial or complete conversion to large cell carcinoma morphology. Conversion to large cell carcinoma was accompanied by loss of APUD cell markers including L-dopa decarboxylase, formaldehyde induced fluorescence, neurosecretory granules, and polypeptide hormone secretion. Preliminary analysis by flow cytometry indicates that most cultures retain their characteristic aneuploid peaks, while others may acquire new aneuploid peaks. Morphological change is also accompanied by increased cloning efficiency, faster doubling time, and increased substrate adhesion. Squamous cell differentiation has also been seen in one culture, with prominent bundles of tonofilaments present in cells retaining neurosecretory granules. We interpret these changes as indicative of a unitarian origin of all lung cancers from an endodermal stem cell. Because SCBC have APUD cell differentiation, loss of these properties is interpreted as progressive loss of differentiation, analogous to squamous cell and adenocarcinomas. We are attempting to generate similar changes in clonal cultures, and will determine whether morphological change is accompanied by alterations in drug sensitivity.

3.103 Calcitonin Secretion by cultures of Small Cell Bronchogenic Carcinoma (SCBC). Drs. Gazdar, Carney, and Drs. Becker, Silva, and Snider, Metabolism Laboratory (WVAH).

SCBC patients frequently have elevated levels of serum calcitonin. Immunoheterogeneity studies have demonstrated that the sera of these patients contain multiple fractions of immunoreactive calcitonin(iCT), with a preponderance of a large molecular form. We have undertaken a survey of iCT secretion by continuous cultures of SCBC.. Using a mid-portion antibody, we have demonstrated that nine of twelve (75%) of cultures secrete iCT. While multiple fractions were present in the supernatant fluids, the predominant form was high molecular weight. In contrast, analysis of the heterogeneity pattern of a calcitonin secreting culture revealed that the principal fraction was monomeric calcitonin. Addition of ^{125}I -labeled calcitonin to cultured SCBC cells did not result in formation of high molecular weight iCT, suggesting that the latter may be formed during secretion. These in vitro studies confirm the high incidence of iCT secretion by SCBC cultures, and demonstrate that the extracellular heterogeneity pattern may not reflect that present intracellularly. The SCBC cultures offer useful models to study hormone secretions by tumor cells. Future studies will explore iCT secretion by clonal cultures, and loss of iCT secretion in long term cultures, especially those undergoing morphological change to large cell carcinoma.

3.104 Characterization of hormone production and clonal heterogeneity of continuous cultures of small cell carcinoma of the lung. (Drs. Radice, Gazdar, Minna and Dr. Dermody, FCCR).

Several recent reports reveal the production of multiple hormones by small cell carcinoma of the lung in culture. In an attempt to characterize our small cell continuous cultures we developed in collaboration with the Frederick Cancer Research Center radioimmunoassays for ACTH, calcitonin, and arginine vasopressin. Nine of our initial lines were grown up in sufficient quantities to provide media and pellets for testing. After 24 hours in serum-free media the supernatants and pellets were collected for radioimmunoassay. In our initial screening 5/9 lines were positive for ACTH and calcitonin production, while 1/9 lines were positive for AVP production. In 7/9 lines at least one hormone was produced. Two of these lines were cloned in soft agarose and propagated./ Supernatants and pellets were collected from these clones in a similar manner. These clones revealed significant heterogeneity in both the expression and production into the media

of these hormones. One line which showed no detectable levels hormone production in mass culture had clones which produced calcitonin and/or AVP. This clonal heterogeneity may explain the observed differences in hormone content of tumor metastases seen in vivo and the problems encountered in the use of these hormones as tumor markers in patients.

We plan to continue to use these radioimmunoassays to aid in the characterization of new small cell lines as well as newly developed clonal lines from several of the established lines. The hormonal products provide an excellent biologic marker to correlate with other small cell characteristics including morphologic changes, cell surface antigens, enzyme content of cells, and growth requirement. The clones of these lines also provide a means of investigating the regulation of hormone production.

3.2 Long term in vitro propagation and characterization of cultures from cutaneous T-cell lymphomas. (Drs. Gazdar, Carney and Bunn, E. Russell, Chemist, Dr. Schechter, WVAMC, Dr. Guccion, WVAH, and Drs. Gallo, Poiesz, Ruscetti and B. Haynes, NIH).

As previously described by us, mitogens may be used for the propagation of malignant T-cells. Cutaneous T-cell lymphoma (CTCL) cells are more resistant to the toxic effects of mitogens than normal T-cells, and selective proliferation of the former occurs in the presence of a suitable mitogen (choice of mitogen is determined by pretesting using thymidine uptake tests). Using these methods, we have established and characterized two continuous cell cultures from patients with CTCL. Several other CTCL specimens have propagated for limited periods of time (about 12 weeks), and even such temporary cultures have been used for a variety of experiments. The two continuous cultures have the same properties as the CTCL cells present in the patients they were derived from, including E-rosette formation, but they lack B cell markers and EBV virus. Thus, they are useful in vitro systems for the study and characterization of CTCL. The cultures release several lymphokines, and one of them is tumorigenic in athymic nude mice.

3.201 Isolation of a retrovirus from NCI H-102 cells.

Type-C virus particles retrovirus were isolated by Dr. Gallo's Laboratory) from one of the cultures, NCI H-102, and from T-lymphoblastoid cells from the same patient (patient C.R.). The NCI H-102 cells now constitutively produce the virus and these are an important source of all viral studies. The virus appears to be a unique virus, different from those isolated from other species. Of interest, a similar virus was isolated from another CTCL line established by Dr. Gallo's laboratory. In collaboration with

Dr. Gallo and his co-workers, we are attempting to determine the frequency with which the virus is present in CTCL patients and their relatives, and the relationship of it (if any) to the disease. In this connection, Dr. Gallo's laboratory has been characterizing the virus isolated biochemically and immunologically. They have found antibodies in our CTCL patient C.R. which indicate the virus is not a laboratory artifact.

3.202 Monoclonal antibody characterization of CTC Cells.

Several subsets of T-cells are known to exist, and in collaboration with Dr. Barton Haynes, we are attempting to subtype CTCL cells. Dr. Haynes has supplied us with 5 monoclonal (hybridoma) antibodies raised against human T cells. Our testing indicates that some of the antisera have HLA specificities, while others react with all T-cell cultures, or only with some T cell cultures. Unfortunately, none react specifically with CTCL cells. We will continue to try and identify antibodies with specificity towards CTCL cells. Such antibodies would be useful for the study of the biology of CTCL, and also for the diagnosis and quantitation of small numbers of CTCL cells in skin, blood, lymph nodes, and other organs.

3.203 Mitogen response of circulating cells in patients with cutaneous T-cell lymphomas. (Drs. Carney, Bunn and Gazdar in collaboration with Dr. Schechter, Hematology, WVAMC).

The response to mitogen stimulation of peripheral blood mononuclear cells from 14 patients with CTCL was compared with that observed in 13 healthy volunteers. In all patients the majority of circulating cells were atypical lymphocytes (Sézary cells). Responses to mitogen stimulation was measured by the incorporation of tritiated thymidine ($^3\text{H-TdR}$) and by flow cytometry. A wide concentration range of 5 mitogens (phytohemagglutinin, concanavalin A, pokeweed mitogen, lymphocyte conditioned medium and staphylococcal protein A) were tested. Responses were tested 3, 5 and 7 days after plating cells in RPMI 1640 medium supplemented with 20% fetal bovine serum. CTCL cells demonstrated a wide range of responses to mitogen stimulation ranging from non-responsive to normal responses. In contrast to normal lymphocytes which demonstrated maximum responses to the T-cell mitogen PHA, CTCL cells were poorly responsive to this mitogen and to lymphocyte conditioned medium, a medium selective for the growth of normal T-cells. Pokeweed mitogen and staphylococcal protein A were the most potent mitogens for CTCL cells suggesting that these cells may have arisen from a particular subset of T cells having the same responses. Peripheral blood mononuclear cells, total lymphoid cells from a lymph node effaced with tumor cells, and E-rosetted cells from this node, obtained from a single patient were evaluated for their mitogen responses. All three populations of cells gave

identical responses suggesting that Sezary cells in different sites share common membrane properties and thus may be monoclonal in origin. Cytogenetic studies confirmed that malignant aneuploid cells were responding to pokeweed mitogen and staphylococcal protein A in contrast to PHA which selectively stimulated "normal" cells. This suggests that future cytogenetic studies should be performed using these mitogens in lieu of the commonly used PHA. We conclude that CTCL cells may represent the malignant expansion of subsets of T cells which can be recognized by the pattern of their responses to mitogen stimulation, as well as by specific antibodies. The lymphocyte transformation assay may be useful means for identifying specific growth factors for CTCL cells.

3.204 Mitogen Responses of Circulating Normal and Malignant Cells: A Comparison of Response Measured by Tritiated Thymidine Incorporation and Flow Cytometry. (Drs. Carney, Bunn and Gazdar).

Studies of the proliferative responses to mitogenic stimuli routinely use tritiated thymidine ($^3\text{H-TdR}$) as a radiolabel. The major disadvantage of this method is that information about transformation of individual cells or subpopulation of cells is lacking and results are therefore valid only when a homogenous population is tested. Flow cytometry (FCM) provides very rapid and quantitative information about the DNA content, RNA content, proliferative patterns, etc., about a given cell population and where two or more populations of cells exist (diploid and aneuploid) can provide proliferative data for each population. We investigated the use of FCM in measuring the proliferative responses to a variety of mitogens of peripheral blood mononuclear cells from healthy controls and 10 patients with cutaneous T-cell lymphomas and correlated this with the results obtained using $^3\text{H-TdR}$ incorporation. A significant correlation was observed for both controls ($r=.94$) and for CTCL patients ($r=.91$) between results measured by both methods. FCM provided the advantage of direct measurement of the DNA content per cell and allowed the identification, where mixed populations of diploid and aneuploid cells exist, of which population was responding to mitogen stimulation. We conclude that as the proliferative response to mitogen stimulation of both normal and neoplastic lymphocytes measured by either $^3\text{H-TdR}$ incorporation or by FCM are significantly correlated, the value of FCM in identifying the specific cell population responding to mitogens by aneuploidy, in samples with mixed populations of normal and neoplastic cells makes it a useful additional technique for measuring lymphocyte blastogenesis in response to a variety of stimuli.

3.3 Growth and characterization of human tumors heterotransplanted into athymic nude mice. (Drs. Gazdar and Carney, and H. Sims, Biologist).

We have established and maintained several transplant lines of human tumors in athymic nude mice. These tumors provide bulk amounts of human tumor tissues for biological and immunological studies, are useful for testing chemotherapeutic and other treatment regimens, and have been used as sources for the establishment of continuous in vitro cultures. In addition, nude mice have been used to confirm the tumorigenicity of established human tumor cultures, and to determine the histological appearances of the tumors so induced. Injection of tumor cell colonies from human tumors plated into soft agarose into the brains of athymic nude mice has resulted in tumor induction in several cases, confirming the tumorigenicity of the colonies, and demonstrating that the cells have the same cyto-morphology as those present in the original human tumor.

Special emphasis is placed on small cell bronchogenic carcinoma (SCBC) as well as non-SCBC lung cancer. We have established and maintained over 10 serially transplanted human lung cancers. While the non-SCBC tumors maintain their morphology over several transplant generation, SCBC tumors gradually change their morphology to large cell undifferentiated carcinoma. As described previously with cultures, loss of SCBC morphology is associated with loss of APUD cell features. This has major implications as SCBC transplants are being used to screen new antineoplastic drugs. The transplanted tumors are currently being used to test antineoplastic drugs, and to compare tumor growth rates with those in the patient and in continuous cell culture. In addition to lung cancer transplants, we have successfully transplanted an osteogenic sarcoma and a renal cell carcinoma. The osteogenic sarcoma elaborates much bone, and it has been established in culture. Injection of the cultured cells into nude mice results in a bone forming tumor. It offers a useful model to study bone formation in vitro and in vivo. A renal cell carcinoma, established in continuous culture as well as serially transplanted, is morphologically identical to the original clear cell carcinoma. Mice bearing the transplanted tumor invariably waste away, and die when their body weight is reduced by about 50%. We have not noticed this with any other transplanted tumor of human or animal origin. The carcinoma cells are apparently secreting an as yet unknown substance which induces fatal cachexia. We will continue to establish and characterize human tumors in nude mice.

3.4 Clonogenic Assays for Human Tumor Cells Obtained Directly from Patients (Drs. Carney, Gazdar, Bunn and Minna)

Tumor cells from many different histologic types have been shown to clone in a semi-solid medium when obtained directly from patients. A good correlation exists between the presence of tumor cells in the biopsy specimens and the growth of colonies in agar suggesting that only tumor cells will grow in this system to the exclusion of non-malignant cells. Evidence exists that this clonogenic assay may be used for predicting sensitivity and resistance to chemotherapeutic agents for individual patients. There is still little data on the cloning of human lung cancer cells in this system. We have developed methods to clone SCCL cells and to optimize cloning conditions. In addition, we have developed methods whereby we can rapidly increase the number of tumor cells available for plating in the assay system thereby allowing this assay system to be used in patients with SCCL for the selection of therapy.

3.401 Development and Optimization of a Clonogenic Assay for SCCL (Drs. Carney, Gazdar and Minna).

The methods used for clonogenic assays are modifications of those described by Hamburger and Salmon and co-workers. Using their plating system we found that the colony forming efficiency for established SCCL cell lines to be 10-100 fold less than in RPMI 1640 culture medium. Evaluation of the many individual factors in the Salmon system indicated that horse serum, DEAE dextran suppressed the CFE of the SCCL cells. In addition, the CFE in both CMRL 1066 and McCoy's medium was shown to be inferior to that observed using RPMI 1640 medium. Testing of many other media demonstrated no advantage over RPMI 1640 medium. The optimal concentration of fetal bovine serum with the cell lines tested ranged from 10-20%. From the results of these studies we are presently plating fresh human tumor specimens of SCCL in 0.3% agarose in RPMI 1640 culture medium supplemented with 20% fetal bovine serum over a base layer of 0.5% agarose in culture medium which has hardened.

3.402 Results of Clonogenic Assays for Freshly Isolated SCCL Cells (Drs. Carney, Gazdar, Bunn and Minna).

Freshly isolated specimens were obtained from 91 patients with SCCL. The sources of the specimens were as follows: bone marrow aspirates (60); pleural effusions (15); lymph node biopsies (9) and liver biopsies obtained under direct vision at peritoneoscopy (7). Thirty-six specimens had pathological evidence of SCCL and 55 were negative. Colony formation was observed in 32/36 specimens positive for SCCL and in 7/55 specimens negative for SCCL. In SCCL positive specimens

colony formation was observed 4-7 days after plating and reached their peak size (100-1000 cells) 7-10 days after plating. Deterioration in colonies was observed 14-21 days after plating. Attempts to feed colonies with additional medium did not significantly improve the survival of the colonies in agarose. The number of colonies per plate (per plated 10^5 viable mononuclear cells) ranged from 3-262 (mean 66.6/plate, median 22) yielding a colony forming efficiency per plated mononuclear cells of 0.003-0.26%, very similar to that reported for other human tumor specimens. The number of SCCL tumor cells in each specimen was determined by examination of cytology preparations of specimens obtained immediately prior to plating in agarose and by calculating the number of aneuploid cells in each clinical specimen as determined by flow cytometry. This allowed the calculation of the CFE per plated SCCL tumor cell which ranged from 0.05-1.5%, very similar to that observed with established SCCL cell lines. The origin of the colonies in these cultures was confirmed by cytologic examination of multiple colonies from each positive specimen. All colonies revealed typical SCCL morphology.

Colony formation was also observed in 7 specimen pathologically negative for SCCL. In 3 specimens colonies with the typical morphologic appearance of SCCL colonies (tight spherical aggregates of several hundred cells) were observed. Although the clinical specimens processed for pathology were negative for SCCL, examination of the cytology preparation obtained immediately prior to plating in agarose revealed typical SCCL cells in each specimen suggesting that sampling problems had accounted for the initial discrepancy. In the 4 remaining specimens with colony formation, colonies very different to typical SCCL colonies were observed. These colonies were mixtures of diffuse and compact colonies typical of hematopoietic stem cell colonies. Cytological examination of these colonies did not reveal any cells with SCCL characteristics. Instead, large mononuclear cells were observed which were immature members of the granulocytic series. The neoplastic cell origin of typical SCCL colonies was also confirmed by flow cytometry examination of pooled colonies from 7 positive specimens. In instances where the clinical specimen revealed both a diploid and aneuploid population of cells, FCM analysis of pooled cells from 10-20 colonies revealed only a single aneuploid population of cells, with a DNA content identical to that observed in the original clinical specimen.

In 18 specimens histologically positive for SCCL, the influence of 25% conditioned medium from a rapidly growing, established SCCL cell line (NCI H69) was tested. In 10 specimens the CFE was increased 1.3-2.0 fold. Suppression of the CFE was not observed in any specimens. In addition to improving the CFE, in the majority of specimens cultured with CM the growth rate and size of individual colonies was markedly increased. From these studies we conclude: 1) SCCL tumor cells can

be cloned directly from patients with a CFE of 0.003-0.26% of the mononuclear cells plated and up to 1.5% of the SCCL cells plated; 2) The origin of these colonies can be confirmed by cytological examination and by flow cytometry; 3) There is an excellent correlation between the formatin of colonies and the presence of tumor cells in pathologic specimens; 4) The occasional growth of SCCL colonies in specimens negative for SCCL suggests that this assay may be a useful means for detecting the presence of tumor cells in clinical specimens; 5) Conditioned medium from established SCCL cell lines may increase the CFE of tumor cells in many specimens. These studies form the basis for testing the sensitivity of SCCL in vitro to chemotherapeutic agents.

3.403 The "Stem" Cell Nature of Clonogenic Cells in Agarose (Drs. Carney, Gazdar and Minna)

We attempted to determine if clonogenic SCCL cells were truly stem cells by examining their ability to demonstrate continuous replication when transferred to liquid medium and by innoculating colonies intracranially into athymic nude mice. The results obtained with colonies from fresh tumor specimens were compared with established SCCL cell lines handled in an identical manner. None of 135 individually picked colonies from fresh tumor specimens demonstrated the ability to continuously replicate when transferred to liquid medium in contrast to 21/26 colonies of established SCCL cell lines which continued to grow. All cell lines were tumorigenic in nude mice. We have presently inoculated pool colonies (1-12 per specimen) intracranially into athymic nude mice from fresh tumor specimens. The latent period to tumor formation is 8-12 weeks. All animals developed signs of intracranial tumor. One animal has thus far been sacrificed and histological examination of the brain revealed typical SCCL infiltration of the meninges and brain parenchymal tissues. Cells from this intracranial tumor cloned in agarose with a CFE of 0.8%. In addition, cells were placed in tissue culture and have remained proliferating for 3+ months.

The inability of colonies from fresh human tumor specimens to demonstrate continuous replication when transferred to liquid medium, in contrast to colonies from established cell lines would suggest that either 1) these cells are not truly "stem" cells with continual "self-renewal capacity" or 2) that our culture system lacked specific growth requirements for the continuous growth of these cells. The latter is more likely as these colonies were tumorigenic when transfered into nude mice.

The ability of colonies from fresh human tumor specimens to form tumors in nude mice confirms their neoplastic origin. In addition, as these tumors clone with a high efficiency in agarose after nude mouse transplantation this may be a means whereby sufficient cells may be obtained for an in vitro drug sensitivity testing when the number of tumor cells and number of colonies formed from the fresh specimen is insufficient to carry out the assay.

3.404 Increasing the Number of Tumor Cells Available for in vitro Drug Testing (Drs. Carney, Gazdar and Minna.)

A major problem associated with the in vitro clonogenic assay and particularly associated with SCCL is that the number of tumor cells in each specimen available for plating is usually insufficient to carry out in vitro drug testing. We have attempted to increase the number of tumor cells by initially culturing specimens in a serum free medium supplemented with growth factors that support the growth of established cells lines in the absence of serum (vide infra). Normal cells cannot replicate in the serum free, hormone supplemented medium while SCCL tumor cells do. We have cultured 7 positive SCCL clinical specimens, 3 lung adenocarcinoma specimens and one melanoma. Immediate and rapid proliferation of SCCL tumor cells to the exclusion of all other cells was observed in all 7 SCCL specimens within 24-48 hours of plating in culture. Cells from one of these specimens was cloned after 48 hours culture in serum free medium and cloned in agarose with a CFE of 0.2%. While the number of tumor cells in the original clinical specimen contained insufficient cells for in vitro drug testing, after 2 days culture in serum free medium sufficient cells were available for testing many drugs. Only one of the human lung adenocarcinomas grew in this serum free medium while the melanoma failed to do so. The selection process of this serum free medium (SHITE medium) in promoting rapid initial proliferation of SCCL tumor cells to the exclusion of "non-malignant" cells, and the ability of these cells to the exclusion of "non-malignant" cells, and the ability of these cells to clone in agarose suggests that this may be a means for increasing the number of tumor cells available for in vitro drug testing when few tumor cells are present in the original pathological specimen. Alternatively, this may prove to be a superior method for isolating tumor cells compared to the agar system.

3.405 Clonogenic Assay for Non-SCCL Human Tumors (Drs. Carney, Gazdar and Minna).

The ability of a variety of other human tumors (excluding SCCL) to clone in agarose was tested using the same assay as outlined for SCCL. Thirty specimens were tested including adenocarcinoma of the lung (10), parathyroid adenomas (5), prostate (2); melanoma, insulinoma,

hypernephroma, mesothelioma (2) etc. Colony formation was observed 50% of the specimens plated and the number of colonies per plate ranged from 16-860. No colony formation was observed in the parathyroid adenomas. Colonies formed with lung adenocarcinoma were morphologically different from those observed with SCCL and cytological examination of these colonies revealed only cells with the cytological characteristics of adenocarcinoma. Colonies from the single melanoma plated and one lung adenocarcinoma have been transplanted into athymic nude mice. The period since inoculation has been insufficient to determine if tumor formation will occur.

3.406 In vitro Drug Sensitivity of Human Tumor Cells. (Drs. Carney, Gazdar and Minna.)

The problems with in vitro drug testing for SCCL have been previously outlined. The major problem is that the vast majority of specimens contain too few tumor colony forming units. We have outlined methods which should, in part overcome this deficit and allow in vitro drug testing to be carried out for SCCL. Thus far we have carried in vitro drug sensitivity testing in 6 patients: 3 with SCCL; 2 with lung adenocarcinoma and one patient with malignant melanoma. Sufficient colony formation for evaluation was present in only three specimens. Resistance to the drugs tested in vitro was also observed in vivo.

We have also used established SCCL cells to determine the activity of phase I agents.

We plan to prospectively select chemotherapy for patients with SCCL who have failed their initial regimen of combination chemotherapy based on the results of the in vitro clonogenic assay. This will provide a test for the usefulness of the assay.

3.5 Identification of Growth Factor Requirements for Lung Cancer Cells in Chemically Defined Media (Dr.s. Abrams, Gazdar and Minna).

Identification of the specific growth factor requirements of human lung cancer cells provides invaluable insights into the biology of these cells which may permit the modulation of their proliferation both *in vitro* and, ultimately, *in vivo*. An ideal cocktail of hormone and growth factor requirements may, in addition, enable us to take advantage of the unique ability of neoplastic cells to grow in the absence of serum for drug testing. We have defined the growth factor and hormone requirements for established cell lines of small cell bronchogenic carcinoma which allow them to proliferate in completely defined, chemical media without serum and to replicate continuously for a year. The assays we have used have been: 1) growth curves in liquid media in microwells and 2) flow microfluorometry to determine the fraction of cells synthesizing DNA. We have recently developed, but not as yet applied, a third method which involves an anti-HLA monoclonal antibody, detected by I^{125} labeled anti-mouse antibody, which we have demonstrated binds to cells grown in microtiter wells as a function of the cell number. The utilization of this technique will enable us to test multiple cell lines under a variety of conditions simultaneously. The growth curves, however, provide the most complete and reproducible data and will continue to be employed as the ultimate test for cell proliferation. These curves are performed in tissue culture media (usually RPMI-1640) to which are added single or multiple growth factors, and compared to unsupplemented media and 10% fetal bovine serum. A cocktail of five factors have been identified (insulin, 10ug/ml, transferrin 10 ug/ml, hydrocortisone $10^{-8}M$, and selenium 3×10^{-8} , which permits the continuous replication of small cell bronchogenic carcinoma cell lines in the absence of serum for at least one year; stimulates the growth of these lines over RPMI alone but somewhat less vigorously than 10% FBS; maintains their tumorigenicity in nude mice and the ability to clone in soft agarose without serum; and retains the original properties of the parent SCBC cells with regard to neurosecretory granules, dopa decarboxylase activity, and DNA content. Many other factors (including ACTH, blood meal, calcitonin, ceruloplasmin, decadron, FSH, fibroblast growth factor, EGF, glucagon, G-H-L, human growth hormone, LH, LHRF, MSA, NGS, prolactin, progesterone, parathormone, somatostatin, putrescine, TS, TRH, TSH, testosterone, and AVP) have been tested with little or no improvement in the growth rate and some have been frankly inhibitory. Deletions from the SHITE formula have been made on cells which had been adapted to it for many months and only transferrin was noted to be absolutely necessary for prolonged proliferation. Cells have been maintained for at least six months in selenium, insulin and transferrin alone with growth rates not significantly different from SHITE. In addition, one line has been isolated which can grow in the absence of insulin, which Sato and others have claimed to be an absolute requirement for serum-free cell growth. Clearly

these cells need to be studied and compared with parent cells for small cell characteristics, growth with and without insulin and insulin receptors. The search for the optimal formula, testing other growth factors and combinations, must continue with the ultimate goal of hormonal growth modulation detected in vitro and in heterotransplanted nude mice to be applied in patients

3.6 Genetic Analysis of the Malignancy of Human Lung Cancer Using Hybrid Cells and Other Gene Transfer Techniques. (Drs. Alvarez, Oie, Gazdar and Minna)).

Somatic cell hybridization is a useful technique for genetic analysis of malignancy. Hybrid cells formed between malignant and non-malignant parents may be analyzed for malignant markers such as tumorigenicity in homologous hosts or nude mice replication in low concentrations of serum, and for the ability to form colonies in semi-solid media. The retention or loss of specific human chromosomes can be correlated with expression of the malignant phenotype or its suppression by gene products of the non-malignant partner.

Unexpectedly we have found that it is very difficult to generate viable, replicating hybrids between lung cancer cells and normal rodent cells. Because of this, we have invested time in systematically studying the fusion conditions for generating viable hybrids using these new techniques. Our laboratory has now successfully hybridized NCI-H-64 and NCI-H-128 clone 2 small cell lung carcinomas and adenocarcinoma of the lung (A549 clone 8) to rodent cell lines (BHK Hamster and BRL 30 E Rat). These hybrids have revealed that small cell carcinoma chromosomes are selectively and preferentially eliminated from the hybrid cell line, and thus we are able to search for specific human chromosomes which code for the characteristics of lung cancer cells. Because BRL 30 E is non-tumorigenic malignant characteristics expressed in the lung cancer X BRL 30 E hybrids can be correlated with chromosomes of the malignant human small cell lung cancer lines.

Recent advances in gene transfer technology have included transfer of chromosomes, chromosomal fragments, nuclear fragments, and isolated DNA using coprecipitates with calcium phosphate. This has allowed other investigators to transform non-malignant cells with DNA from malignant cells. We have begun such experiments using DNA isolated from lung cancer cells. We plan to assay experiments using DNA isolated from lung cancer cells. We plan to assay for both transfer of malignant characteristics, and markers of interest in small cell lung cancer including polypeptide hormones.

3.7 Mapping of Human Genes using Somatic Cell Hybrides, (Drs. Alvarez, Oie, Radice, Gazdar, Cuttita, Minna).

In addition to the use of hybrids for identifying the chromosomes which code for malignancy, our laboratory plans to determine which code for the production of the polypeptide hormones of ACTH-endorphin, calcitonin and argenine-vasopressin, as well as the APUD marker L-dopa decarboxylase and the fraction of creatinine phosphokinase. All of these markers are expressed by our small cell carcinoma lines.

We are fusing clonal lines of small cell carcinoma which have been characterized for the production of these polypeptide hormones and other APUD markers to a variety of rodent cells, some of which are related to the APUD system of differentiation but which do not express these proteins. We are first establishing which markers are expressed dominantly and then we will follow the expression of the markers with chromosomal segregation.

The monoclonal antibodies against human tumor cells which mark cell surface antigens can also be used to follow the expression of these antigens in human tumor x rodent cell hybrids. The question of dominance or recessivity of these antigens, and their chromosomal localization will be of general biologic interest. In addition, other monoclonal antibodies which react with all human cells but not with Chinese hamster or mouse cells can be used to follow these antigens in hybrids cells. These monoclonal antibodies thus provide a new set of genetic markers and eventually a panel of such antibodies will permit rapid screening or immunoselection of hybrid cells with a desired human genetic content.

Other potential uses of hybrid cells made with human lung cancer include their use as immunogens in the production of monoclonal antibodies. By immunizing mice with mouse x human lung cancer hybrids that have segregated all but a few human chromosomes the number of human antigens the mouse is exposed to can be dramatically reduced. This approach has been useful in the generation of monoclonal antibodies against melanoma antigens.

Many drug resistant markers are dominantly expressed in hybrid cells. It is thus theoretically possible to create stable human x human hybrids made between continuously replicating human cell lines of known drug sensitivity and human tumor cells taken directly from patients. We are trying to produce such hybrids as a continuing source of material for in vitro drug sensitivity testing as well as a source of cell surface antigens.

3.8 Production of monoclonal antibodies using somatic cell hybrids (hybridomas) with specificity for human tumor cells (Drs. Minna, Cuttitta, Abrams, Radice).

3.801 Development of immunoassays to detect antibody production.

We have been immunizing mice of various strains with our human small cell carcinoma line (NCI H-69C), fusing the mouse spleen cells to mouse myeloma cells (P3X63, RGNS1 and 653) and testing the resulting hybrids for antibody production using a radioautography in 96 well microtiter plates. Our detection system utilizes as little as 5×10^4 target cells per well and gives a signal to noise ratio of $10 \times -30\times$ for positive responders, with backgrounds under 100 cpm. The target cells are glutaraldehyde fixed to the polyvinyl plate and nonspecific binding sites are blocked by coating with 1% BSA. Hybridoma immune products are screened against several small cell lines (NCI lines H-69e, H-128 and H-187), B-cell lines (NCI H128BL, NCI H-51 and NCI H-77), T-cell lines (NCI H-1012, NCI H-78, CCRF-HSB2, CCRF-CEM and RPMI-8402), large cell line (NCI-H231), neuroblastoma cell lines (IMR-32), adenocarcinoma cell line (A549), human lung cell line (VA2), mouse cell line (RAG and B82) and Chinese hamster cell line (E36).

We have developed a simple but rapid way of sampling hybrid supernatants directly from the culture plate using a 96 well diluter (25 ml aliquot). The diluter allows one to sample multiple times from the same culture plate and test supernatants against our battery of target cells. Supernatants are transferred and routinely diluted 1:5 in the target cell plate. I^{125} labelled goat anti-mouse IgG (affinity purified) is used to detect hybridoma antibody bound to target cells. To shorten exposure times during the radioautographic procedure, we have incorporated a combination of Kodak XR-5 film and Dupont Lightening plus intensifying screens. Using the screening technique described above we can process 5000 or more samples in 48 hours.

3.802 Production of monoclonal antibodies with specificity for small cell lung cancer (Dr. Cuttitta, Dr. Minna, and Dr. Radice).

Hybrids from the primary fusion are initially screened against NCI H-69c, NCI H-128 and NCI H-128BL to evaluate their selective response for small cell carcinoma. Hybrids which give a positive reaction with small cell but not on autologous B lymphocytes on the preliminary trial are minicloned to a 96 well plate and the resulting progeny retested for selectivity. The miniclone

technique offers a rapid way of isolating selective hybridoma lines and at the same time gives a measurement of the line stability. The stability of any given hybrid is clearly ascertained after repeat miniclone. Since stable lines give a homogeneous response over the entire plate following miniclone.

We have screened over 10,000 cultures and detected antibody activity in approximately 10% of the hybrids. Of these, approximately 1% appear to have specificity for lung cancer cells. We now have a stable hybridoma line which will react with small cell but not autologous B lymphocytes. The antibody has also given preliminary results which verifies its inability to react with A549 and SV 40 transformed embryonic lung cells VA2.

In order to maximize the number of hybrids expressing selective antibody, we have been studying the effect of multiple immunization schedules and examining the variation in immune response to small cell carcinoma of several strains of mice (CAF, SJK/J, BALB/C, and DBA/2J). We are hopeful that standardization of protocols will increase the efficiency of selective hybrid production and will set the foundation for this laboratory's further development of tumor specific monoclonal antibody.

3.803 Production of Monoclonal Antibodies Against Non-Small Cell Lung Cancer Cells, (Drs. Abrams and Minna).

Production of highly specific, unique antibodies to cell surface antigens of non-small cell lung cancer cells would permit the isolation of differential and possibly tumor-specific antigens. Clinically, they may be employed for the identification of pathological sub-sets which may have different behavior; the early detection of tumors at resectable, and therefore, potentially curable states; and as an adjunct to therapy either as cytotoxic antibodies to specify cells or as a means of directing other (e.g., drug) treatments directly to the tumor.

We are currently engaged in the production of monoclonal antibodies against adenocarcinoma of the lung cell lines. These cells were taken directly from a patient with adenocarcinoma of the lung and grown in tissue culture. They are injected into mice or rats, and after appropriate boosting doses are given, the animal is sacrificed and its spleen harvested and then fused to myeloma cells. The hybrids are grown in selective HAT media in microwells and the supernatants tested for antibody production against the same adenocarcinoma cells fixed to plastic plates with I¹²⁵ labeled anti-mouse antibody used to detect bound antibody with auto-radiography. Human B cells are used as the initial control to demonstrate specific binding to differentiation, as opposed to HLA antigens. We have currently produced at least one, and possibly three, antibodies which appear to react specifically with adenocarcinoma cells. Further testing of this antibody, as well as cloning and stabilizing the cells which are producing it is currently underway. Characterization of these antibodies shall proceed while additional antibodies are produced. Development of an epidermoid lung cancer line will permit the production of monoclonal antibodies against this form of lung cancer. In conjunction with the other work being performed in our laboratory, the cell surface antigens recognized by these antibodies can be isolated and compared across the different histological types of lung cancer. Somatic cell hybridization between lung cancer and other cell lines, which is also underway in our laboratory, will also permit chromosome assignment of the human genes responsible for the particular cell surface antigens isolated and whether or not they are dominantly expressed. Since human-non-human hybrids segregate human chromosomes, any monoclonal antibody which reacts with the human cell and the hybrid, but not the other cell line, will define a human antigen on a human-non-human hybrid.

3.9 Production of monoclonal antibodies using the somatic cell hybrids (hybridomas) with specificity for hormones and other products secreted by lung cancer cells. (Drs. Radice and Minna).

Lung cancer cells frequently secrete polypeptide hormones. ACTH, endorphin, arginine vasopressin, calcitonin, and their precursor forms have been isolated from fresh tumor specimens as well as tumor cells lines. Other neuroendocrinologic hormones, including substance P, neurotensin, bombesin, and somatostatin, which are produced by cells that may share embryologic origins with small cell carcinoma have not been investigated as possible tumor markers. Monoclonal antibodies with specificity for these hormones that could be produced in abundance would be valuable not only for general endocrinology but also as reagents to study the regulation of hormone production in vitro, and the possible role as tumor markers in vivo. To this end, we have begun immunizations with these hormones in multiple mouse strains. The spleen cells from these immunized mice are then fused with mouse plasmacytoma cell lines to form hybrids capable of producing specific monoclonal antibodies against the immunizing hormone. To date we have investigated several different immunization schemes and found significant differences among mouse strains in their response to these immunogens. To test the hybrid clones for hormone production, we have developed a sensitive microtiter plate assay. As little as 10 ng of hormone bound to the well can detect specific antibody production using labeled protein A or rabbit-anti-mouse immunoglobulin in an autoradiographic system. Using this assay system we have identified several specific clones to ACTH in initial screening. However, there has been difficulty in maintaining activity over several clonings. To enhance the stability of these initial hybrids, we are not using the technique of mini-cloning multiple times prior to using the limiting dilution method for obtaining single clones.

We feel that the hybridoma technique is extremely important in producing new immunologic reagents, and we plan to continue this work, characterize the antibodies so produced, and then use them in radioimmunoassays for characterizing the peptide hormones produced by lung cancer cells both in tissue culture and in patients.

3.10 Production of Monoclonal Antibodies Using Somatic Cell Hybrids (Hybridomas) for Specific Proteins Including Myoglobin and Lysozyme. (Dr. Minna and J. Fedorko in collaboration with Dr. Brezofsky MET/NCI)

Using hybridoma techniques, monoclonal antibodies against sperm whale myoglobin antibodies and eggwhite lysozyme were produced in tissue culture. Anti myoglobin antibodies from several ones were tested and found to produce linear Scotchard plots, as expected for homogeneous antibodies, and to possess high affinities for the immunogen ($K_A=10^9 M^{-1}$). Characterization of the antibodies revealed that 5 of the 6 tested were of the Class IgG1 while the sixth was IgG2a. Nine (75%) of 12 anti sperm whale monoclonal antibodies bound to equine myoglobin, while 2 of 6 monoclonals bound to human myoglobin with affinities within 3-fold that for sperm whale myoglobin. This suggests that these monoclonal antibodies recognize different antigenic determinants on the myoglobin molecule. Studies using additional myoglobins from several species where primary amino acid sequences of the myoglobins are known will allow a precise definition of the determinants. Those reactive will have clinical application while the others will be useful in studying the evolution of proteins.

3.11 Establishment of Companion B Cell Lines to Tumor Lines for Use in Monoclonal Antibody Production. (Drs. Oie, Gazdar, Carney and Minna).

Monoclonal antibodies to a large number of tumor cell and differentiation antigens are continuously being generated. Such antigens should be present only in tumor and not in most normal cells. Most important is the screening procedure in sorting hybrids producing antibodies reacting with all human cells from those producing selective reacting antibodies. HLA and other histocompatibility antigens are immunodominant and thus many "selective" antibodies detect HLA differences. The solution to this is to use normal cells (autologous) from the same patient the tumor cell line derived thus avoiding HLA differences. However, it is not always possible to obtain normal cells for antibody testing; and of course, this is impossible in cases where the patient has passed away. An easy, feasible solution to this problem is to establish B cell lines from each patient at the time tumor cell cultures are being started. B cells can be "transformed" by infecting them with the Epstein-Barr virus and then establishing permanent B cell lines. These B cell lines can then be maintained in culture and will be conveniently available for the testing of monoclonal antibodies as they are produced. Antibody which react with the tumor cells but not with their companion B cells could be regarded as a possible antibody to a tumor specific antigen.

3.12 Establishment and Characterization of a Rat Islet Tumor Cell Line and Clones Producing Insulin, Somatostatin, and Glucagon. (Drs. Oie, Gazdar, H. Sims, Biologist; Dr. Chick, Harvard Med. Sch.; Dr. Weir, Virginia Commonwealth U.; Drs. Bhathena, Smith, and Recant, Diabetes Res., WVAMC).

Functioning islet cells from human and other species have been maintained as mixed cultures for varying periods of time. However, the limited in vitro life of these islet cell cultures necessitates preparation of fresh cultures for each experiment. This could be time-consuming and costly. We have established a continuous line, RIN-m, of islet cells from a transplantable rat insulinoma. These cells are epitheloid, hypodiploid, contain typical insulin and somatostatin granules, produce tumors in nude mice, and have APUD properties, including formaldehyde induced fluorescence and DOPA decarboxylase activity.

By using media fortified with conditioned medium from a rat pituitary tumor line GH3 which produces prolactin and growth hormone, a large number of primary clones have been isolated from RIN-m. The clones segregated into 4 groups: clones which secrete both insulin and somatostatin; clones which secrete only one of the two hormones; and clones which did not secrete either of the hormones. After 15 or more passages in culture, 13 of 14 clones, including 8 which initially secreted only one of the two hormones, secreted both hormones. From a primary clone C1-5, an insulin secretor, subclones of only 3 phenotypes were isolated: subclones which secreted both hormones; subclones which did not secrete either of the hormones; and a large number which produced insulin only. No subclone producing somatostatin alone was isolated. From clone CL-14, a somatostatin secretor, subclones of only 2 phenotypes were generated: subclones which produced both hormones; and a large number of somatostatin producers. No subclone secreting insulin only was isolated. These studies indicate the insulinoma contains stem cells capable of differentiating along several pathways of hormone production. At least a portion of these pathways are reversible. These clones provide new tools for studying the steps in differentiation of APUD cells

A number of agents designated as secretagogues increase hormone secretions by islet cells. RIN-m and its clones and subclones also respond similarly to such secretagogues as glucose, arginine, theophylline, and elevated K^+ and Ca^{++} . It was during such studies that glucagon was also found to be produced by these cells. The effects of arginine and K^+ on glucagon secretion by the parent RIN-m cells, a high insulin secretor C1-5F, a high somatostatin producer C1-14B, and a high glucagon secretor C1-41 were compared. Arginine, a potent secretagogue for glucagon, increased glucagon secretion by all 4 cultures, but C1-41 secreted 1.5 times more glucagon than the other cultures. High

K⁺ treatment produced an inhibition of glucagon secretion by the parental RIN-m cells, a response similar to that found when perfused whole pancreas was treated with K⁺ (Bhathena, Perrino, Voyles, 1976), but stimulated glucagon secretion by all 3 of the other cultures, notably in the non-insulin producers, C1-41 and C1-14B. The differences in glucagon secretion between the parent RIN-m cells and its clones in response to high K⁺ treatment suggest that paracrine effects may be involved. These cultures provide important tools for the study of mechanisms controlling hormone secretions by the different islet cells.

Specific receptors for insulin, somatostatin, and glucagon have been demonstrated to be present on RIN-m cells. Specific binding for all 3 hormones was linear with cell number and maximal at 1 hour. Bound Insulin and somatostatin were dissociable and "down regulated" with increased hormone concentrations. Analyses of Scatchard and affinity plots of parental RIN-m high insulin secreting subclones, and high somatostatin producing subclones showed that the highest number of receptors for insulin and somatostatin were found on high somatostatin producers, while receptor numbers were similar in parent and high insulin secreting subclones. The presence of specific receptors for all 3 hormones in parent and subclones alike support the likelihood that hormone regulation among the islet cells is probably mediated by all 3 hormones.

Studies on receptors and secretagogues to better understand islet cell hormone secretions will be continued. The clones secreting hormones will be used for production of hybrid cells for gene mapping of hormones, receptors, and APUD properties.

3.13 Establishment and Characterization of a Human Pheochromocytoma Cell Line. (Drs. Oie, Gazdar, H. Sims Biologist and L. Reid, Albert Einstein U.)

An established pheochromocytoma cell line PC-12 of rat origin has long been available for study. Now, we have a human pheochromocytoma line as well. We have started a culture from a transplantable human pheochromocytoma #342 (noradrenalin, adrenalin, and dopamine). The pheochromocytoma cells, free of fibroblast contamination, have been growing in culture for nearly 3 months. These epitheloid cells grow in tightly packed colonies which double in population about every 48 hours. A high concentration of insulin (1-10 ug/ml) appear to be required for optimal growth. These cells have the APUD property of fluorescing when exposed to formaldehyde vapors. We have had some success in our early attempts to clone this cell, and have 5 clones in early culture. Further characterization of this cell will be done, especially with respect to hormone secretion. This cell makes available another human cell line for the study of APUD properties. In addition, these cells could be used for gene mapping of the catecholamine metabolizing enzymes.

3.14 Use of somatic Cell Hybrids as Cloning Vehicles for Specialized Products. (Drs. Minna, Oie, Alvarez, Radice, Cutitta).

Prior work from our laboratory has shown that differentiated functions from normal cells can be expressed in replicating hybrid cells if a tissue culture parent is chosen that is differentiating along the same pathway of the desired normal cell parent. Our prior work had been in the expression of neural crest properties in mouse neuroblastoma cells fused to cells from the normal nervous system. While the best example of this from other work is of course the production of monoclonal antibodies by myeloma x normal B cell hybrids. We are trying to extend this by fusing APUD derived tumor cell lines from man and mouse including small cell lung cancer, pheochromocytoma, insulinoma, and pituitary tumors to normal cells of the APUD system and then scoring the resulting hybrids using radioimmunoassays for the production of new polypeptide hormones. Thus, we are trying to use the tumor cell line as a vehicle for cloning a new function from the normal tissue cell which by itself cannot replicate in culture. This approach should have far reaching consequences in medicine and biology for generating novel cell lines and their protein products. At least some of the products we will be testing for include peptide hormones that can regulate the growth of human lung cancer cells.

3.15 Laboratory Studies of Tumor Blood Flow (Dr. M. Cohen).

The hypothesis tested in these studies is that decreased capillary blood flow will cause decreased delivery of nutrients, oxygen and chemotherapeutic agents to tumor cells. Capillary blood flow is primarily determined by the ability of red cells to deform since red blood cell diameter often exceeds the diameter of the capillary. Evidence that capillary flow to tumors is marginal at best is indicated by the fact that the internal milieu of a tumor has a higher CO_2 tension, lower pH, higher lactic acid concentration and lower oxygen tension than normal tissue.

Chemotherapy responsiveness is determined by pretreatment tumor burden. Treatment of intravenously transplanted L1210 leukemia with optimal single doses of cyclophosphamide or BCNU resulted in greater than 50 per cent cures when the tumor burden was 8×10^5 cells. When the tumor burden was 8×10^7 cells, however, the cure rate decreased to less than 5 per cent for both drugs. For Lewis lung carcinoma optimal single dose BCNU or cyclophosphamide treatment on day 2 after intravenous inoculation of 10^6 tumor cells produced 50 and 80 per cent cures, respectively. When treatment with these two drugs was delayed to 14 days after tumor transplantation no cures were achieved. In human neoplasia it is difficult to precisely measure tumor burden. Estimations of tumor cell mass within the body can be made, however, by determining the number of organ systems involved by cancer. In patients with diverse neoplasms, including small cell lung cancer or female breast cancer, patients presenting with multiple organ system involvement by tumor have a significantly worse prognosis than do individuals with diseases confined to one or two sites.

In this laboratory it has been demonstrated both in murine test systems (L1210 leukemia and Lewis lung carcinoma) and in patients with small cell lung cancer that red blood cell deformability decreases with increased tumor burden. In L1210 leukemia red cell deformability was normal for the first 4 days after intraperitoneal transplantation of 10^5 viable cells. During days 5 to 9 after tumor transplantation, however, red cells become significantly more rigid. Decreasing deformability was associated with a rapid decline in hematocrit, presumably because poorly deformable red cells were cleared from the circulation. In Lewis lung carcinoma red cell deformability became abnormal 14-21 days after transplantation.

Patients with small cell lung cancer also had decreased red cell deformability with advancing disease. There was no difference in deformability for patients with chest only disease or for chest disease plus an additional metastatic site. When patients had chest disease plus two or three additional sites, red cell deformability was significantly decreased.

3.1501 Correction of Red Cell Deformability Abnormalities (Dr. Cohen)

Two methods for improving red cell deformability have been found. The first involved treatment of L1210 leukemia bearing mice with red cell metabolic substrates including inosine, adenosine, glucose, sodium pyruvate or ascorbic acid. The combination of inosine plus sodium pyruvate (3 mg of each drug in 0.5 cc phosphate buffered saline) was most effective in restoring deformability to normal. Administration of an active chemotherapeutic agent (BCNU or cyclophosphamide) also improved red cell deformability with maximal restoration occurring 4-5 days after drug treatment. Standard, or 50 per cent of standard drug doses were equally effective in restoring deformability.

Therapy based on improved red cell deformability (Dr. Cohen)

BCNU alone cannot cure mice when treatment is delayed 7 days after tumor transplantation. The optimal therapy program for day 7 L1210 leukemia utilized inosine plus sodium pyruvate given 10-15 minutes before BCNU 15 mg/kg on day 7 and before BCNU 30 mg/kg on day 12. This treatment yielded 44% cures whereas BCNU alone, in identical dose and schedule gave no cures. Median survival was 50 days for the inosine-pyruvate treated mice versus 30 days for BCNU alone. Therefore, treatment with non-toxic doses of red blood cell metabolic substrates plus optimal timing of chemotherapy, two maneuvers that significantly increased red blood cell deformability, resulted in significant therapeutic benefits.

3.1502 Toxicity of chemotherapy related to red cell deformability. (Dr. Cohen)

Toxicity of intensive induction chemotherapy was compared in small cell lung cancer patients with decreased red cell deformability versus patients with normal deformability. The dividing line was the lowest level of deformability observed in normal controls. Patients with decreased deformability had significantly greater weight loss ($p = 1.0005$), required more red cell transfusions ($p = 0.025$), more platelet transfusions ($p = 0.05$) and had more days at less than 1000 white blood cells ($p = 0.025$). In addition, 6 of 7 patients who had bilateral pulmonary interstitial disease on this treatment had abnormal red cell deformability.

3.16 Chemotherapy Induced Diffuse Interstitial Pulmonary Disease: An experimental model. (Drs. M. H. Cohen and M. J. Matthews).

Diffuse interstitial pulmonary disease associated with anti-neoplastic therapy is an important cause of treatment related morbidity and mortality. A murine model has been developed. Chemotherapeutic agents studied thus far include adriamycin, mitomycin C, BCCNU and cyclophosphamide. Adriamycin did not cause pulmonary toxicity while the latter 3 drugs did. Pathologic changes included moderate to severe vascular congestion, interstitial pneumonitis, pulmonary hemorrhages, pulmonary edema, and alveolar cell metaplasia. The primary determinant of pulmonary toxicity was tumor burden at the time treatment was initiated. Toxicity was not noted when treatment was given 5 days after L1210 transplantation but occurred in 100% of animals when treatment was delayed to days 7 or 8 after transplant. Endothelial damage appeared to be the primary event with epithelial injury occurring in smaller numbers of animals.

Experimental Prevention of Diffuse Interstitial Lung Disease. (Dr. Cohen).

Measures that improve red cell deformability delay and ameliorate pulmonary toxicity from chemotherapy. Treatment of L1210 bearing mice 3 days after tumor transplantation or Lewis lung cancer 21 days after transplantation with cyclophosphamide 250 mg/kg resulted in 100% and 50% mortality respectively from pulmonary toxicity. Pretreatment of mice with drugs that improve red cell deformability including pitressin, inosine-pyruvate and dextran decrease mortality to 50% in L1210 leukemia and to 0 in Lewis lung carcinoma.

3.17 Experimental models of Adriamycin cutaneous toxicity. (Dr. Cohen).

Skin necrosis following subcutaneous Adriamycin extravasation is a significant clinical problem. In this study Adriamycin dilutions and various drugs were tested for their ability to ameliorate this toxic effect. When Adriamycin was diluted to a concentration of < 0.25 mg/ml, no skin ulceration was observed. Drugs such as hydrocortisone, dexamethasone, lidocaine, bupivacaine, phentolamine, and hyaluronidase were ineffective in modifying Adriamycin skin necrosis. Care in IV drug administration and dilution of Adriamycin are the best means of preventing the sequelae of subcutaneous Adriamycin extravasation.

3.18 Laboratory studies on the kinetics of human malignancies. (Drs. Bunn, Carney, and Gazdar)

3.1801 Kinetic studies in lung cancer. (Dr. Bunn)

We have shown that the majority of small cell tumors namely 31 out of 39 tested directly from patients are aneuploid whereas 9 of 10 non-small cell tumors were aneuploid. In all instances the aneuploid cells were hyperdiploid and in 3 of the 48 patients with aneuploid tumors there were more than one stem line. We have then compared the DNA content of the same cells grown in liquid tissue culture and in athymic nude mice. In all instances these cells growing in these culture conditions had the same DNA content as those in the patient. The DNA content of the non-small cell tumors were shown to be higher on the average than the small cell tumors, however, these differences are not yet statistically different. We have shown in the patients with multiple stem lines that cloning the cells can separate these cells into single stem lines. We have compared the results of flow cytometry with standard pathology using either cytology of histology and have shown that in most samples taken from any site other than bronchial washings that there is an excellent correlation between flow cytometry and pathology. In a few instances cytology is positive when flow cytometry is negative and in a few instances flow cytometry is positive when cytology or histology are negative. These show that the two methods are complimentary and discrepancies are felt to be due primarily to sampling problems when small numbers of malignant cells are present in a sample. However, there are false negative flow cytometric results in 10 to 20 per cent of samples when the tumors are diploid. We have recently purchased a dual parameter cell sorting system which will hopefully allow detection of these diploid tumors using dual parameter flow cytometry studies. In addition, we have analyzed the cell cycle distributions in the lung cancer tumors from both patients and from the tissue culture systems. In addition, we have been measuring the doubling times of the tumors in patients in liquid tissue culture and soft agar system in vitro and in heterotransplants in nude mice. These studies are ongoing at present. It does appear however, that the doubling times of small cell tumors with a median of about 88 days are shorter than the median and mean doubling times of the non-small cell tumors, which range from 95 to 180 days. In the nude mice heterotransplants it also appears that the doubling times in small cell tumors are shorter than the non-small cell tumors. The cell cycle distributions in the patients have not been found to be markedly different between small cell tumors and non small cell tumors however, most of the non small cell tumors which have been studied are large cell carcinomas. In a literature review of labeling index status, the labeling index is generally highest in small cell lung cancer, next highest in large cell lung cancer and patients with epidermoid and adeno lung cancers have much lower labeling indices. We have

found that the fraction of cells in S phase is markedly similar in different metastatic sites within the same patients and that the fraction of cells in S are similar in primary sites and in metastatic sites. This is in contrast to some data in the literature suggesting that the S fraction is higher in metastatic sites than in primary sites. We have also been analyzing the cell cycle distribution patterns in our in vitro system where it does appear that the small cell lung cancer cell lines have higher fractions of cells in S than the non-small cell lung cancer cell lines. In addition, it appears that in the nude mouse heterotransplant the small cell carcinomas have a shorter doubling time than the non-small cell tumors in the nude mice.

These studies are currently ongoing.

We have recently purchased a new multiparameter cell sorter and plan to analyze our lung cancer cells both in vivo and in vitro using dual parameters in order to detect those cells which are diploid by DNA content. We are also instituting studies of the drug perturbation effects of cytotoxic chemotherapeutic agents on lung cancer cells grown in these in vitro systems.

We, in collaboration with Dr. Brian Brigham have shown that the labeling index in patients with mixed small cell large cell carcinomas is higher in the large cell component than in the small cell component and that in small cell carcinomas the labeling index is higher in large malignant cells than in smaller malignant cells. These studies were performed using tritiated thymidine and autoradiography.

3.1802 Kinetic studies in cutaneous T-cell lymphomas. (Drs. Bunn, Carney and Gazdar.

We have shown that by cytogenetic studies essentially 90 per cent of patients have aneuploidy. About 15 per cent of patients have marked hyperdiploidy and all of these patients can have their hyperdiploidy detected by flow cytometry. These patients have a worse prognosis than patients with diploid or near diploid tumors. A large fraction of patients have cytogenetic abnormalities with near diploid DNA contents. These are manifest by a wide CV when performing studies with flow cytometry. Patients with such a wide CV have a worse prognosis than those with no detectable abnormalities by flow cytometry. In addition, we have shown that patients with large fractions of cells in S phase have a worse prognosis than those with low S fractions. However, patients with large S fractions are much more likely to be hyperdiploid. In two patients with aneuploidy we have demonstrated that cells in the lymph nodes have a higher S fraction than cells in the skin and that cells in the

peripheral blood have a very low S fraction. We have shown that in tissue culture flow cytometry with calculation of the cells in S the response to growth factors including T-cell growth factors can be studied.

We have shown that when histologic conversion from small cell variants of T-cell lymphomas convert to large cell variants of T-cell lymphomas this is caused by a change from diploid or near diploid to hyperdiploid DNA content and is associated with a poor prognosis much the same as histologic conversion from small cell to large cell in B cell lymphomas are associated with a poor prognosis. We have also shown that the fraction of cells in S as calculated by flow cytometry following mitogenic stimulation of the malignant cells gives identical results of those using in vitro titrated thymidine.

3.19 Immunologic studies with flow cytometry in cutaneous T-cell lymphomas. (In collaboration with Drs. Dean Mann, Barton Haynes and Anthony Fauci, Laboratory of Clinical Investigation, National Institutes of Allergy and Infectious Disease and in collaboration with Dr. Ivor Royston, University of California, San Diego).

We have shown that the T-cells from our patients and our T-cell lines do not express the 3A1 monoclonal T-cell antibody which is present on the majority of normal T-cells.

Thus, all or nearly all the malignant T-cells are 3A1 minus. The malignant T-cells do have high amounts of a 4F2 monoclonal antibody which is associated with rapid proliferation of human cells. We have shown that the malignant Sezary cells in these patients, however, do have the ability to help normal immunoglobulin synthesis in vitro. Normal T-cells bearing the 3A1 monoclonal antibody help in vitro immunoglobulin synthesis. This suggests that our malignant T-cells which help immunoglobulin synthesis but which do not bear the 3A1 antibody either are a monoclonal expansion of a 3A1 negative clone which can help or which were initially 3A1 positive cells which during malignant transformation lost the expression of the 3A1 antibody.

In collaboration with Drs. Haynes and Royston we are undertaking the study of other monoclonal T-cell antibodies.

3.20 Kinetic Studies in Multiple Myeloma. (Dr. Bunn)

We have shown that flow cytometry is more useful in demonstrating aneuploidy in patients with multiple myeloma than is cytogenetics. At diagnosis about 50 per cent of patients with myeloma can be shown to have hyperdiploid tumors

and these patients are more likely to have advanced stage and have a significantly worse survival than patients with diploid tumors at diagnosis. Patients with initially aneuploid tumors who achieve a response have a diminution in the aneuploid fraction of cells and patients having a complete remission have a total loss of the aneuploid fraction of cells. However, with relapse the aneuploid clone reappears. In one patient this aneuploid clone reappeared in the bone marrow prior to clinical relapse. At the time of relapse more than 80 per cent of patients have aneuploid tumors and this is associated with a very poor prognosis. Because the aneuploid tumors have a mean and median DNA content 1.2 times normal using single parameter flow cytometric studies it is impossible to determine the S fraction in the malignant cells. However, in collaborating with Dr. Oliver Alibaster from the Laboratory of Pathology, National Cancer Institute we have shown that dual parameter studies allow separation of malignant cells have aneuploid DNA contents. This can allow for determination of the S. fractions in both the normal and in the malignant cells. Further dual parameter studies are now in progress with our new dual parameter cell sorter. In addition also in collaboration with Dr. Alibaster we have published a literature review of the cell kinetic studies in multiple myeloma.

3.21 Kinetic Studies in other Human Malignancies.

We are using flow cytometry to examine specimens from a variety of other cancer including ovarian carcinoma, prostate carcinoma, other malignant lymphomas and solid tumors.

3.22 Laboratory Studies of Megakaryopoiesis (Dr. Bunn in collaboration with Dr. Levine, Hematology, VAMC)

We have shown that cytometry provides rapid and accurate measurements of megakaryocyte ploidy.

Using this technique we have shown that 8N is the most frequent ploidy class in guinea pig marrows. We have also shown that monkeys have higher ploidy values than guinea pigs. We have shown that procedures designed to enrich megakaryocyte numbers introduce a bias by selecting megakaryocytes with high ploidy values. We have tested a new integrated microdensitometry, the Artek 800 image analyzer and shown that megakaryocyte ploidy values measured using this instrument are similar to those measured in the Coulter TPS 1 cell sorter. We are currently measuring megakaryocyte ploidy values in humans and in rats and

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PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Study and Treatment of Cancer in the Young

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

25

20

15

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Acute leukemia, Non-Hodgkin's lymphomas (e.g. Burkitt's), neuroblastoma, rhabdomyosarcoma, osteosarcoma, Ewing's sarcoma and aplastic anemia are studied. In leukemia, we have devised therapeutic regimens which may allow maximum tumor cell kill while minimizing sequelae. Emphasis is on tailoring treatment to individual prognostic variables, and improving the therapeutic ratio in CNS prophylaxis. In the solid tumors, we have developed combined modality approaches to primary disease. In refractory tumors, we are studying the utility of high dose-therapy and the role of supportive care (platelet and white cell transfusion, laminar-flow protection, autologous bone marrow rescue, total parenteral nutrition) in permitting such therapy. Phase I-II trials are also conducted in refractory tumors; agents include ICRF-187, poly I:C/poly-l-lysine, dihydroxyanthracenedione, and 2'-deoxycoformycin. We are exploring the biology of selected tumors, including kinetics, immunology, virology, tumor markers, genetics, biochemistry, and pharmacology. Also studied are the effects of cancer and its treatment on growth, development, and organ function. The psychosocial concomitants of cancer are explored.

Objectives:

1. To devise effective therapeutic regimens in acute leukemia which allow maximum tumor cell kill with drugs and radiotherapy, while leaving intact effective elements of the immune response and minimizing therapeutic sequelae -- especially in the CNS.
2. To develop effective combined modality treatment approaches to previously untreated childhood solid tumors, including rhabdomyosarcoma, neuroblastoma, non-Hodgkin's lymphoma, osteogenic sarcoma, and Ewing's sarcoma.
3. To study the possible role of high-dose ("ablative") chemotherapy in advanced solid tumors, and the role of supportive care (platelet and white cell transfusions, laminar-flow protection, bone marrow rescue, hyperalimentation) in permitting such ablative therapy.
4. To study the biology of selected tumors, including kinetics, immunology virology, prognostic markers, karyotypes, biochemistry, and drug sensitivity.
5. To assess the pharmacology of selected new agents, e.g., high-dose methotrexate, dihydroxyanthracenedione, ICRF-187, poly I:C/poly-l-lysine, 2'-deoxycoformycin, and lphosphamide in humans.
6. To study the short- and long-term effects of malignant disease and cancer treatment on growth, development, and organ function.
7. To study the psychosocial concomitants of cancer in young patients, their families, and their therapists.

Methods and Major Findings:A. Therapeutic Regimens for Acute Lymphocytic Leukemia (ALL)

1. Protocol 72-1 - was instituted as a consequence of Mathe's report in 1969 suggesting a beneficial effect of immunotherapy in childhood ALL. The 72-1 study was designed to compare the effectiveness of chemotherapy plus immunotherapy versus chemotherapy alone as maintenance treatment for newly diagnosed patients with this disease. The study was designed to expose patients to immunotherapy early, following remission induction, rather than after long periods of chemotherapy. Remission induction therapy consisted of repeated five day courses of POMP (prednisone, vincristine, methotrexate, and 6-mercaptopurine). All patients were then treated with systemic consolidation chemotherapy and central nervous system prophylaxis. The latter consisted of 2400 rads to the whole brain and intrathecal medication given during the period of cranial radiation and monthly thereafter for

30 months. Patients were randomized to receive one of two types of intrathecal chemotherapy, either methotrexate or cytosine arabinoside. Following completion of consolidation chemotherapy and cranial irradiation, patients were randomly assigned to a 34 month course of maintenance treatment. Patients received either chemotherapy alone, or chemotherapy plus immunotherapy with non-irradiated, live allogeneic leukemia cells and BCG. Immunotherapy was given periodically throughout maintenance during two month periods during which no chemotherapy was given. A total of 70 patients were entered on this study which was closed to entry in 1975. Sixty-three patients (90⁰/o) achieved complete remission. There has been no significant difference in the relapse rate between those patients who received chemotherapy alone and those who received chemotherapy plus immunotherapy. Similarly, there was no significant difference in remission duration between the maintenance treatment arms. At the present time 27 patients (43⁰/o) are in their initial complete remission, having completed therapy and off all treatment. Five patients have been off therapy for two years, seven for three years, four for four years, and eleven for five years. Six of 33 patients (18⁰/o) have relapsed after completing therapy. In terms of prognostic factors, it is notable that 55⁰/o of the patients who entered this protocol had an initial white blood count >25,000 per mm³ and/or were less than two or greater than ten years of age. Eighty percent of the relapses have occurred in patients with poor initial prognostic signs. In contrast, 80⁰/o of those patients currently in unmaintained remission were in a good prognostic category initially.

2. Evaluation of the Long-Term Effects of CNS Prophylaxis - The institution of central nervous system prophylaxis (cranial radiation and intrathecal methotrexate) has radically altered the treatment of ALL. Not only has it reduced the incidence of meningeal leukemia from 50-70⁰/o to 5-10⁰/o, but it has also been responsible for a marked improvement in bone marrow remission duration and increased long-term survival. Nevertheless, attention has been directed to the possible adverse effects of CNS preventive therapy. Several POB studies have highlighted the potential dangers of the cranial radiation plus intrathecal methotrexate utilized in the current standard approach to CNS prophylaxis.
 - a. Abnormal Computed Tomography Brain Scans in Children with ALL following CNS Prophylaxis - A serious form of delayed neurotoxicity which has been observed in ALL patients treated with cranial radiation and either intrathecal or systemic methotrexate is necrotizing leukoencephalopathy. The clinical manifestations of this syndrome include confusion, forgetfulness, dysarthria, dysphagia, dementia, ataxia, spasticity, seizures, coma and death. The syndrome has also been observed in patients treated without cranial irradiation,

or with other forms of intrathecal chemotherapy. Usually, however, the leukoencephalopathy has been preceded by cranial irradiation administered in conjunction with intrathecal or systemic methotrexate. In 1978, we evaluated 5 patients with the clinical syndrome of leukoencephalopathy by computed tomographic brain scanning and found distinctive CT scan abnormalities. The ability of computed tomography to detect findings consistent with the pathologically characteristic lesions of this syndrome suggested that such scanning might be a useful modality for monitoring patients who had received CNS prophylaxis. Consequently we recalled patients on the 72-1 protocol who had received CNS preventive therapy for computed tomographic brain scanning. In this study, 32 asymptomatic patients with ALL who had received prophylactic cranial radiation (2400 rads), and (by randomization) either intrathecal methotrexate or cytosine arabinoside, were evaluated by computed tomography 19 to 67 months after the initiation of prophylaxis. Seventeen of 32 (53%) had one or more abnormal findings. Dilatation of the ventricles (8 patients) and widening of the subarachnoid spaces (9 patients), suggestive of brain atrophy, were equally distributed among patients in both intrathecal chemotherapy groups. Areas of decreased attenuation coefficient (hypodense, abnormally radiolucent regions) (4 patients) and intracerebral calcification (one patient) -- lesions previously described in methotrexate leukoencephalopathy -- were found only in those individuals who had received intrathecal methotrexate. Although minor neurologic abnormalities were detected in some patients in the study group (see below), they did not correlate with the CT scan abnormalities. The unexpectedly high prevalence of such abnormalities contrasted with the essentially normal tomographic findings in a control group of patients with acute lymphocytic leukemia who received no CNS prophylaxis. The results of this study heightened concern over the potential adverse effects of CNS preventive therapy and suggested that alternative approaches to such prophylaxis should be sought.

- b. Neurological and Psychological Evaluation of ALL Patients Who Have Received CNS Prophylaxis -- We have performed a comprehensive neurological evaluation of the 32 apparently asymptomatic patients discussed above. Abnormalities of both the peripheral and central nervous system were detected. Peripheral neurological findings (decreased deep tendon reflexes, distal lower extremity motor weakness, altered vibratory sensation, and paresthesias) were found in 19 patients. These findings are probably secondary to vincristine. In addition, 8/32 patients manifested ataxia; two of these had lateral gaze nystagmus. However, these findings

did not correlate with the presence of CT abnormalities, nor with the particular type of intrathecal chemotherapy. Comprehensive psychological testing has been performed in those patients on the 72-1 protocol who remain in complete remission. Testing has been carried out by Drs. Howard Moss and Ellen Nannis (NIMH). All subjects were administered the Wechsler Intelligence Test and the Bender-Gestalt Test of perceptual motor function. Different forms of the Wechsler test were used for different age groups (WPSI for children between 4 and 5 years; WISC for children between 6 and 16 years; and the WAIS for children over 16 years of age). These intelligence tests were administered to the CNS prophylaxis patients and to 3 control groups. The first control group included 34 siblings of the above ALL patients, with a mean age similar to that of the patients. This group presumably was also similar to the patients in genetic and environmental background. A second control group consisted of 13 surviving ALL patients who had never received any CNS therapy. (Siblings of these patients were also tested). A third control group consisted of 22 cystic fibrosis patients (and their siblings), chosen because they represent a group of frequently hospitalized children. The healthy sibling controls attained significantly higher IQ scores than did the patients who had received CNS prophylaxis. The IQ differences between the CNS treated patients and their siblings were statistically significant ($P < .001$) for comparisons involving the Full Scale, Verbal Scale and Performance Scale IQ's in both the initial and one year follow-up testing. In contrast, the ALL patients who did not receive CNS treatment, and the cystic fibrosis patients (who also did not receive any CNS treatment) obtained IQ scores that did not statistically differ from the IQ's of their respective sibling groups. Analysis of the results also revealed that younger patients had significantly greater decrements in Full Scale and Verbal Scale IQ scores than older patients. The results of this study indicate that CNS preventive therapy with cranial radiation and intrathecal chemotherapy can significantly impair both intellectual and perceptual motor functions. The IQ scores averaged about 94 for the CNS treated ALL patients and about 112 for their healthy siblings. These scores would place the patients at the 47th and their siblings at the 79th percentile ranks. This difference of approximately 13 IQ points between the patient and sibling group is sufficient to have a bearing on their potential for academic achievement. The findings of this study again suggest that it is appropriate to search for alternative methods of CNS preventive therapy.

- c. Neuro-Endocrine Function in ALL Patients Following CNS Prophylaxis - In an attempt to identify possible adverse effects of CNS prophylaxis upon the hypothalamic-pituitary axis, we examined the hypothalamic-pituitary function of 23 ALL patients. Eighteen patients (Group A) had received cranial radiation (2400 rads) and multiple doses of either IT methotrexate or cytosine arabinoside as prophylaxis. The remaining 5 patients (Group B) received no cranial radiation. Patients were recalled for serial determinations of growth hormone, cortisol, thyroid stimulating hormone, T₃, follicle stimulating hormone, luteinizing hormone, and prolactin, following appropriate stimulation. In addition, CT scans were performed within 3 months of the endocrine evaluation. Nine patients in Group A (50%o) had abnormally low growth hormone responses (<7.0 ng/ml). Seven of these 9 patients also demonstrated ventricular dilatation on their CT scans. In contrast, only 1 of the 9 patients in Group A with normal growth hormone responses demonstrated this abnormal CT scan finding. Thus, there was a significant correlation ($P=.015$) between ventricular dilatation on CT scan and abnormally low growth hormone responses. Since ventricular dilatation is believed to result predominantly from cranial radiation, this correlation is consistent with the hypothesis that abnormal GH responses may be due to an adverse effect of radiotherapy upon the hypothalamic-pituitary axis, although a possible contributory role for either systemic or intrathecal chemotherapy cannot be ruled out. It is of interest that both ventricular dilatation and abnormal growth hormone responses have been produced experimentally in animals following high dose cranial radiation. None of the patients in Group B had abnormal CT scans or low growth hormone responses. Since the real test of GH secretion is linear growth, the clinical implications of the abnormal GH responses are unclear. Examination of the linear growth curves in both patient groups did not reveal a correlation between impaired GH responses and short stature. Although eight of the eighteen (44%o) evaluable patients (<12 years old at diagnosis) are in the 10th percentile or less, only four of these eight patients had peak GH responses <7.0 ng/ml, and only one patient's growth curve exhibited a persistent "fall-off" pattern indicative of inhibited growth. However, it is of concern that none of these eight patients has demonstrated "catch-up" growth following the termination of their treatment. "Catch-up" growth may be a more appropriate clinical indicator of normal hypothalamic-pituitary function. It is notable that study of a group of Ewing's sarcoma patients who had received less intensive CNS prophylaxis (2000 R and only a single dose of IT methotrexate) revealed neither CT scan nor neuroendocrine

abnormalities. These data suggest that the presence of CT scan and neuroendocrine abnormalities is related to the intensity of the CNS prophylactic regimen.

3. Protocol 75-1 - Retrospective analysis of protocol 72-1 had demonstrated that patients could be divided into good and poor prognostic groups on the basis of certain "front end" factors. These include age at diagnosis, initial white blood cell count, presence of meningeal leukemia (prior to the institution of CNS preventive therapy) and failure to achieve remission with four induction courses of POMP therapy. In the 75-1 study, patients were segregated on the basis of these previously identified prognostic factors. Patients in the good prognosis group were treated conservatively with moderate dose chemotherapy on a regimen similar to that utilized in the 72-1 protocol. Patients in the poor prognostic group were treated with a slightly more intensive chemotherapeutic regimen which utilized an early intensification course of L-asparaginase and cyclophosphamide and a late intensification course (approximately 6 months after induction of remission) with cytosine arabinoside and daunomycin. Twenty of the 21 patients achieved complete remission. However, 10 of the 16 patients of the poor prognosis group have relapsed in contrast to all 5 patients in the good prognosis group who remain in complete remission. Because the projected remission duration and survival data in the poor prognosis group patients was no different than that observed in our earlier 72-1 poor prognosis patients, the study was terminated at an early stage.
4. Treatment of Newly Diagnosed Acute Lymphoblastic Leukemia - Current Protocol (POB 77-02) - The current ALL treatment protocol addresses the two major therapeutic problems which emerged from the aforementioned NCI 72-1 and 75-1 studies: 1) The need for improved treatment for patients possessing certain poor prognostic factors, and 2) improvement in the method of delivering CNS preventive therapy such that effective treatment can be administered with less morbidity. The main objective of this protocol is to investigate the efficacy of high-dose, protracted intravenous methotrexate infusions as central nervous system preventive therapy. Patients are randomized to receive CNS prophylactic therapy either with cranial radiation (2400 rads) plus intrathecal methotrexate, or with high-dose 24 hour, systemic methotrexate infusions. As noted previously, a major stimulus behind the development of this protocol was our increasing awareness of significant toxic sequelae associated with the prophylactic cranial irradiation. The hypothesis to be tested in the present protocol is that CNS preventive therapy using high-dose protracted systemic methotrexate infusions alone is equally effective and less toxic than the current standard form of CNS prophylaxis (cranial irradiation and intrathecal methotrexate).

An additional aim of this study is to assess the utility of an intensified systemic maintenance schedule which alternates standard maintenance treatment with periodic "induction-type" chemotherapy regimens. The rationale for using the systemic methotrexate approach rather than local therapy to prophylax the CNS is based on several considerations. First, there are ample data which demonstrate that intravenously administered methotrexate can cross into the CSF and achieve therapeutic CSF levels. We have successfully used the proposed intravenous regimen to induce complete remission in overt meningeal leukemia. Second, it is believed that the systemic route is more "physiologic", providing entrance of drug at all levels of the cerebrospinal axis and insuring better drug distribution throughout the CNS including the meninges, the site of origin of leukemic spread to the CNS. Third, moderate dose intravenous methotrexate without cranial irradiation has recently been shown in a randomized study to decrease the incidence of CNS leukemia. Intravenous methotrexate also has been used effectively in the treatment of CNS non-Hodgkins lymphoma. Fourth, intravenous methotrexate has certain technical advantages over intrathecal administration, and obviates the necessity for frequent lumbar punctures. In addition, considerable clinical experience with prolonged intravenous methotrexate infusion and the accompanying citrovorum rescue has shown it to have acceptable systemic toxicity. Pharmacologically, this 24 hour high-dose methotrexate regimen provides more prolonged therapeutic CSF antifolate concentrations than does a standard dose of intrathecal methotrexate both in ventricular and lumbar cerebrospinal fluid. It is hoped that through the use of this methotrexate infusion technique central nervous system preventive therapy can be given which is as effective as, but less toxic than, the combination of cranial irradiation and intrathecal methotrexate. The 77-02 protocol also attempts to provide patients with optimal exposure to those chemotherapeutic agents considered most active against ALL by employing an escalated, intensified maintenance schedule which alternates standard maintenance treatment with frequent, periodic, "induction-type" chemotherapy utilizing varying reinduction regimens. It is hoped that the application of this intensified maintenance schedule will improve the prognosis for "high-risk" ALL patients. Experience to date has shown this intensified approach can be accomplished without a significant increase in drug related morbidity. To date 40 patients have been randomized on this study. Thirty-nine have achieved complete remission (97.50%). Fifteen of these individuals were randomized to receive cranial irradiation plus intrathecal methotrexate, 25 have been treated with the high dose, protracted intravenous methotrexate infusion. (The randomization is weighted on 2 to 1 basis.) There have been 2 CNS relapses (one in each treatment group) and 3 bone marrow relapses (all in the cranial radiation, intrathecal methotrexate arm). These results are not statistically significant; more

patients and additional follow-up time are necessary. Although it is too early to draw meaningful conclusions regarding the utility of the methotrexate infusion arm, the data thus far appear encouraging. It is notable that toxicity associated with the intensive maintenance schedule has been relatively minimal. In mid 1979, two other institutions (The Children's Orthopedic Hospital, Seattle, and the Children's National Hospital Medical Center, Washington, D.C.) joined this protocol as collaborators. This should provide sufficient patient accrual to complete this study within 3 years.

5. Treatment of Acute Lymphoblastic Leukemia in Relapse - Although considerable success has been achieved in the treatment of newly diagnosed, previously untreated patients with ALL, the general experience in the treatment of patients who relapse while undergoing chemotherapy has been far less positive. Our recent experience with an intensive four drug reinduction regimen has, however, been encouraging. Between 1971 and 1976, 125 hematologic relapses of ALL were treated with a 4 drug regimen consisting of L-asparaginase, vincristine, daunomycin and prednisone (Asp-VDP). These relapses occurred in 56 patients whose multiple consecutive relapses were treated with the Asparaginase-VDP protocol. Of the 125 courses evaluated, 114 (91%) resulted in complete remission. Ninety-one percent (51/56) of patients who received their first reinduction course of this regimen attained complete remission. Eighty-nine percent (31/35) of patients also achieved complete remission on their second exposure to this regimen. Although the numbers are small, all of the patients who received 4 or more courses of this regimen for treatment of 4 or more relapses achieved complete remission. Surprisingly, no difference in response rates was noted in patients who received this reinduction regimen as treatment for multiple subsequent relapses, i.e., reinduction efficacy did not decline following repetitive exposures to this regimen. The current relapse protocol (POB 77-09) builds upon our experience with the four drug Asparaginase-VDP regimen discussed above. While remission re-induction rates are excellent, it is evident that more effective maintenance therapy is needed. Protocol 77-09 compares an aggressive maintenance treatment regimen to conventional maintenance therapy. Following induction therapy with the Asparaginase-VDP regimen, all patients receive intensification with cyclophosphamide as well as CNS reinforcement consisting of six weekly courses of intrathecal methotrexate which begin shortly after marrow relapse. Patients are then randomized to receive one of two types of maintenance therapy, an aggressive schedule which involves periodic "re-induction type" treatment alternating with conventional cycles of 6-mercaptopurine and methotrexate, or the conventional cycles alone. This protocol is employed at the time of first and second relapse. Individuals who relapse on one

maintenance arm are re-induced as above, and crossed over to the other maintenance schedule. Thus far 21 patients have been enrolled on the 77-09 protocol. Ninety percent of the 21 patients achieved complete remission. Among these patients there have been 10 relapses. The median duration of remission is 419+ days. The limited accrual to date does not permit us to draw conclusions regarding efficacy of the different maintenance treatments.

B. Therapeutic Regimens for Acute Nonlymphocytic Leukemia (ANLL)

Protocol 78-07 (BCRC) - The present treatment protocol (78-07) is designed to test three forms of maintenance therapy for this disease. Patients who achieve complete remission are randomized to receive either chemotherapy alone, chemotherapy plus splenectomy, or chemotherapy plus immunotherapy. All patients receive identical induction chemotherapy consisting of the combination of cytosine arabinoside (100 mg/M²/day x 7 iv) plus daunomycin (45 mg/M²/day x 3 iv). Patients who achieve remission on this induction regimen are then randomized to one of the three maintenance treatment arms. One-third of the patients receive intensive maintenance therapy every three months with cytosine arabinoside (100 mg/M² iv q 12 h) plus 6-thioguanine (100 mg/M² po q 12 h) given to marrow hypoplasia. A second group of patients receives maintenance chemotherapy (as above) plus immunotherapy with neuraminidase-treated allogeneic blast cells. A third group of patients receives chemotherapy plus splenectomy. This is a BCRC protocol which we have joined. Thus far 52 patients have been entered on this protocol. 67% have achieved complete remission. Eleven have been randomized to chemotherapy alone, eleven to chemotherapy plus immunotherapy, and ten to chemotherapy plus splenectomy. The median remission duration to date for all patients entered on protocol is seven months. Since the median follow-up time is very short, it is too early to make a meaningful assessment of the value of the various maintenance treatment arms.

C. Studies on the Treatment of Overt CNS Leukemia

1. Intrathecal (IT) versus Intraventricular Methotrexate (MTX) in Overt Meningeal Leukemia - We compared intraventricular MTX delivered via the Ommaya reservoir versus IT MTX in the treatment of established meningeal leukemia using each patient as his own control. Ten children who had recurrence of CNS leukemia despite monthly IT MTX were reinduced and maintained with intraventricular MTX. Remission duration with intraventricular MTX was compared with the patient's previous remission duration in association with IT MTX. Seven patients had a longer and one a shorter remission with intraventricular MTX than with IT MTX. Three patients who died of systemic leukemia while remaining in CNS remission on intraventricular MTX had no evidence of CNS leukemia

at autopsy. There were three CNS relapses during 4,009 patient days at risk in the intraventricular group in contrast to 10 CNS relapses in 3,312 days at risk in the IT treatment group. These data suggest that intraventricular chemotherapy is more effective against overt CNS leukemia than IT.

2. Treatment of Overt Meningeal Leukemia with Intraventricular "CxT" Methotrexate - We have evaluated the utility of intraventricular "CxT" methotrexate as treatment for meningeal leukemia. In an earlier study, 19 patients with meningeal leukemia were randomized to receive courses of intraventricular methotrexate via an Ommaya reservoir. These courses consisted of either single injections of 12 mg/M²/dose or a low-dose "concentration x time" (CxT) schedule of 1 mg every 12 hr for 3 days. There were no significant differences between the two treatment groups in the rate of remission induction, the number of relapses, or the durations of remission. The mean (+ 1 SD) cumulative methotrexate dose was 66 + 41 mg/M² in the CxT group and 173 + 64 mg/M² in the 12 mg/M²/dose group (p<0.005). Neurotoxic toxicity occurred in one of the eight patients in the CxT group and in seven of ten patients in the 12 mg/M²/dose group (p<0.05). These observations suggest that the CxT dosage schedule is less neurotoxic and equally effective in the treatment of central nervous system leukemia. We have extended the duration of the "CxT" approach (concentration X time), and 3 patients have received 5 days of CxT methotrexate via subcutaneous Ommaya reservoirs. All patients tolerated the regimen well without overt signs of methotrexate neurotoxicity. The prolongation of the CxT approach to five days allows for maintenance of prolonged therapeutic CSF MTX concentrations and thus theoretically exposes more leukemic cells to this phase-specific agent. We now plan to compare 5 day CxT versus a single intraventricular MTX dose.
3. Treatment with Systemic (IV) Methotrexate Infusions - Our recent studies in patients and in a sub-human primate model have revealed that therapeutic concentrations of MTX can be achieved in cerebrospinal fluid following intravenous infusion. We have evaluated the utility of 24 hour continuous systemic MTX infusions. A relatively constant plasma drug level is maintained for 24 hours; 12 hours later (36 hours after the start of the infusion) citrovorum factor rescue is initiated. The infusion dose over the first hour (the priming dose) is given at a dose of approximately 1.5×10^{-7} times the desired plasma MTX concentration. This is followed immediately by a 23 hour maintenance infusion at an hourly rate of one-fifth the primary dose. Our studies have demonstrated that this technique is feasible and that IV doses which maintain a CSF MTX level of 1×10^{-5} M can be given without apparent systemic or neurological toxicity. We have also determined that the ventricular and lumbar

CSF: plasma MTX ratios are similar, suggesting that systemic MTX administration had the advantage of providing consistent drug concentrations throughout the CSF space. This contrasts with intralumbar administration, which does not provide optimal drug penetration into the ventricular CSF. We have now treated 6 patients with meningeal leukemia and have observed complete responses in all six. This approach will be utilized in our next major meningeal leukemia treatment protocol.

D. Studies on the Treatment of Metastatic Neuroblastoma

We have developed a collaboration (under an international agreement) with the Polish National Institute of Mother and Child to assess Phase II agents in patients with refractory metastatic neuroblastoma. The Warsaw institution is relatively unique in that it treats approximately 45 newly diagnosed cases of neuroblastoma per year. Our initial treatment protocol, begun late in 1978, is designed to test the efficacy of streptozotocin in this disease. Patients selected for study are those who have been demonstrated refractory to established chemotherapeutic agents. Our interest in streptozotocin is based on the known efficacy of this agent against other neural crest tumors. To date, 7 patients have been entered on study. As yet, there have been no therapeutic responses. Nevertheless, this agreement offers a unique resource for the evaluation of Phase II agents in neuroblastoma.

E. Treatment of Metastatic Osteogenic Sarcoma

1. Protocol 77-03 - This study employs total surgical debulking of pulmonary metastatic disease (Phase I) followed by intensive sequential treatment with various agents known to be active in osteogenic sarcoma (Phase II). Following resection, patients are given 1 week of high-dose methotrexate followed by exposure to 3 alkylating agents (L-Pam, DTIC, and cyclophosphamide). During the severe bone marrow hypoplasia which follows such treatment, patients are offered laminar-air-flow isolation, platelet and granulocyte transfusions, and autologous bone marrow rescue where appropriate. Following recovery of peripheral counts, patients are then exposed to 1 week of treatment with adriamycin and cis-platinum. Eighteen months of maintenance with high-dose methotrexate alternating with adriamycin then follow. Patients who cannot be totally debulked at the initiation of this study are treated with high-dose methotrexate (pharmacologic Phase I) in the hope that their lesions will be altered in such a way as to then permit total surgical resection, followed by the intensive sequential chemotherapy described above. To date, we have attempted initial pharmacologic debulking of metastases (Phase I) in 10 patients, and a surgical Phase I in 17. Of the 10 receiving high-dose methotrexate initially, 5 were randomized to receive

repeated 6 hr. infusions and 5 received repeated 42 hr. infusions. Seven of the ten showed no response; an eighth had complete resolution of pulmonary metastases (6 hr MTX) and the other two became operable (42 hr MTX). Of the 17 patients entered on Phase II (intensive chemotherapy in the absence of macroscopic tumor), 15 are evaluable, of whom 10 are free of disease at a median of 24 months (6-40 months) after debulking. It is of interest that the 6 patients who are in remission for at least 18 months after entry on Phase II had at least one episode of pulmonary metastases treated surgically prior to the episode for which they received another debulking and then subsequent treatment on 77-03. Two of these six patients had had three discrete episodes of pulmonary metastases, each separated by no more than a few months, each treated by resection, and each resection followed with various chemotherapies, prior to another debulking and then entry onto 77-03. These two patients are now in continuous disease-free remission at more than 36 and 40 months, respectively, from the beginning of 77-03 chemotherapy. The four patients with one prior episode of metastases had had that episode within a few months prior to the episode that brought them to 77-03, and these four patients are now in remission at 21+, 25+, 28+, and 39+ months following entry onto Phase II of 77-03. Overall, despite the intensive spike of chemotherapy at the beginning of Phase II, the regimen described here has been tolerable with the exception that one patient died during the induction spike from hepatic necrosis of uncertain etiology but probably treatment-related. The maintenance arm of 77-03 includes adriamycin, and approximately 40% of the patients have required that the starting adriamycin dose be reduced at some point because of prolonged neutropenia. Of patients entered on Phase II of 77-03, 60% have experienced continuous disease-free survival. For the reasons previously discussed, it is not possible to say that this represents an improvement over previous experience, since we may simply be seeing the effect of aggressive pulmonary resection per se. On the other hand, the few patients with a past history of multiple episodes of pulmonary metastases, each separated by no more than several months, who now remain continuously disease-free for two or more years following entry onto this protocol comprise an encouraging anecdote, and it is tempting to believe that intensive chemotherapy delivered after extensive debulking of metastases may be an effective approach to the treatment of metastatic osteogenic sarcoma.

2. Protocol 77-08 - Patients who fail 77-03 are given cis-platinum alone, or if they have never received adriamycin, are randomized to cis-platinum + adriamycin. Each monthly cycle consists of 5 days of cis-platinum or 5 days of cis-platinum plus three days of adriamycin. Of the 21 patients entered on the study, 19 are

evaluable. Of these, 10 received cis-platinum alone; 2 stabilized and 8 have no response. Of the 9 receiving both drugs, 6 showed no response and 3 showed stabilization of disease.

3. Protocol 78-02 - To date, 18 patients (Ewing's sarcoma, osteogenic sarcoma, or rhabdomyosarcoma) have been entered on this Phase II study of 5-azacytidine in pediatric sarcomas. There has been only one (equivocal) response.
4. Ancillary study of aryl-hydrocarbon hydroxylase inducibility in the families of patients with osteogenic sarcoma and other malignant diseases - Dr. Daniel Nebert, NICHD, has had a long interest in aryl-hydrocarbon hydroxylase, which functions in humans and other species in the metabolism of hydrocarbons. In an elegant murine model, Nebert has shown that three strains can be bred with heritable aryl-hydrocarbon hydroxylase genetics: In addition to a strain with normal levels of enzyme inducibility, a second strain is excessively inducible with respect to the normal, and a third is not inducible at all. In the strain with no inducible enzyme, exposure to hydrocarbons produces aplastic anemia which eventuates in acute leukemia. In the excessively inducible strain, exposure to hydrocarbons results in the development of sarcomas at the site of hydrocarbon injection. We wondered whether the human genetics of aryl-hydrocarbon hydroxylase might be analogous to those in mice, i.e., whether there might be a tendency toward excessive inducibility of aryl-hydrocarbon hydroxylase in patients with sarcomas and their families, and a relative lack of inducibility in patients with acute leukemia and their families. To approach this question, we have examined enzyme in the peripheral blood lymphocytes of siblings and parents of our patients rather than the patients themselves because of the possible influence of chemotherapy on enzyme inducibility. While we began the study with osteogenic sarcoma families, we have now expanded it to include the families of all patients seen in this Branch, i.e., acute leukemia, lymphoma, and sarcomas. This study has been controlled by examining families who are friends or neighbors of the index families (but not related). The control families are age and sex-matched for each index family. At this time, more than 100 families seen in this Branch have been studied, with an equal number of control families. The study is virtually complete, but the results are not yet available since all specimens will be assayed concurrently.

F. Study and Treatment of Rhabdomyosarcoma

1. Protocol 76-4 (primary treatment) - Previous studies have shown that combination chemotherapy has significantly improved the survival of children with localized rhabdomyosarcoma. Initial therapy for these patients has usually included surgical excision.

However, the sites of tumor involvement (head and neck, GU) often make such surgery mutilating or functionally impairing. Part A of this protocol is designed to assess the utility of initial chemotherapy in "pharmacologically debulking" the primary tumor so as to provide an alternative to "radical surgery." Patients with lesions judged to be surgically resectable are randomized either to initial chemotherapy (followed by radiation and surgery) or to the standard approach of surgery, followed by chemotherapy and radiotherapy. In contrast to the situation with localized tumors, combination chemotherapy has failed to improve the survival of patients with metastatic rhabdomyosarcoma. For such patients, part B of this protocol addresses the utility of intensive (escalating) combination chemotherapy (in conjunction with hematologic and antimicrobial support) versus conventional dose combination chemotherapy. To date, 24 patients have been entered on this study. The median age is 16 yrs (mean 15, range 2-30 yrs). The majority of patients (95%) are Stage II-III. Alveolar histology predominates (15/24), and the primary sites include head and neck (7), genitourinary (7), and extremity (10). Both Stage I patients attained a CR and remain in remission for 24+ months. Eight of the 12 Stage II patients have completed induction therapy and all attained a CR. However, 6/8 have relapsed (median duration 9 mos, range 1-13 mos). Of the 10 Stage III patients, 8 have completed induction therapy. Four of these patients were randomized to the standard dosage chemotherapy schedule and all attained CR. However, three patients subsequently relapsed and died. Six patients were randomized to the intensive drug schedule. One patient died during treatment from radiation-induced pneumonia. Of the 4 remaining evaluable patients, 3 attained CR and 1 patient failed to respond to therapy. Three of the four responders are disease-free, but the time since completion of induction therapy is brief. Of interest is the recent observation of a potential tumor marker. Dr. D. Zopf (NCI Pathology Dept.) has developed a radioimmunoassay for a unique tetrasaccharide breakdown product of glycogen, presumably generated by amylase degradation. Preliminary observations suggest that this material is present in the urine of patients with glycogen-rich tumors (i.e., rhabdomyosarcoma, Ewing's sarcoma). If these observations are confirmed, the assay may prove useful as a biomarker for these tumors.

2. Protocol 75-5 (relapse treatment) - This protocol employs intensive combination chemotherapy administered in escalating cycles for patients whose tumors have demonstrated refractoriness to standard doses of chemotherapy. Seventeen patients have been entered onto this protocol. All patients had evidence of recurrent metastatic disease after relapsing on either the POB 76-4 protocol (see above) or the Intergroup Rhabdomyosarcoma Study Protocol. Of the 17

patients, 2 (12⁰/o) completed only 1 cycle of the protocol, 6 (35⁰/o) completed 2 cycles, 5 (29⁰/o) completed 3 cycles, and 4 (24⁰/o) completed all 4 cycles. Failure to complete the protocol was related to severe toxicity in 2 patients (infection and cardiac failure) and to disease progression in 8 patients. Overall, a complete response (CR) to therapy occurred in 5 patients (29⁰/o) and a PR in 2 patients (12⁰/o). While therapeutic response (CR + PR) was observed in the majority of patients who received three or four cycles of therapy (5/9, 56⁰/o), the duration of response was brief (mean = 4.3 mos, range 1-12 mos). Consequently, while these data suggests that it is possible to deliver very intensive chemotherapy regimens, and while dose escalation appears to overcome the therapeutic refractoriness observed at low dose-schedules, the ultimate utility of such escalating regimens is questionable in this group of patients. Other data does suggest that such treatment schedules may be of more benefit to patients prior to the onset of overt drug resistance; this question is being studied in our primary treatment protocol. In addition, we have evaluated a variety of Phase I and II agents for patients who have failed the primary or relapse protocols. The results have been disappointing, although we have recently observed a possible response to 5-azacytidine in one of four patients treated with this agent. Since 5-azacytidine appears to induce in vitro maturation of rhabdomyoblasts, this response is of interest.

G. Study and Treatment of Non-Hodgkin's Lymphomas

1. Protocol 77-04 (Primary Treatment) - Forty-five patients have been admitted to this protocol since its inception in July, 1977. The major objective is to define prognostic factors in non-Hodgkin's lymphoma. To this end, all lymphomas in children and young adults are treated with an identical chemotherapeutic regimen consisting of repeated cycles of cytoxan, vincristine, adriamycin, prednisone, and high-dose protracted infusions of methotrexate. Tumor cells are obtained whenever possible and studied intensively. Histologically, all are diffuse lymphomas, and have been classified as follows: Burkitt's lymphoma 27, undifferentiated lymphoma 8, lymphoblastic lymphoma 9, histiocytic lymphoma 1. Sixteen patients had limited or entirely resected disease, 29 had extensive intraabdominal or intrathoracic tumor. All except 4 patients have achieved complete remission. The 4 partial responders died, one during intensive therapy from progressive tumor growth, the others after failure of intensive therapy. So far there have been 11 relapses in the group of complete responders. Six of these, all in Burkitt's lymphoma patients, were confined to the CNS, 5 of whom eventually died with resistant CNS disease. The remaining patient is alive after 12 months of unmaintained remission without disease. Five patients had systemic relapses. One had testicular relapse, one a small tumor nodule seen on bone marrow clot

section. The latter patient is currently free of disease following intensive (CARAT) therapy, after 21 months of maintained remission. A third patient had intraabdominal recurrence (undifferentiated) and eventually died with drug resistant tumor; a fourth had local mediastinal recurrence and then bone-marrow relapse, while the final patient (with diffuse histiocytic lymphoma) relapsed with bone marrow and CNS disease. Eleven of 45 patients have died, including 4 partial responders. The overall results, although preliminary, are encouraging and if current trends continue, we would anticipate being able to document a significant improvement over previous treatment. One aspect of these data is the high frequency of isolated CNS relapse in patients with Burkitt's lymphoma in spite of high-dose intravenous methotrexate infusions (6 CNS relapses out of 7 total relapses in 23 complete responders). Although the first two patients to relapse in the CNS had lower doses of methotrexate, this was not true of the remainder. We therefore instituted an intensive prophylactic regimen of intrathecal methotrexate and ara-C (from patient 21 onwards), which, if successful, may result in a dramatic increase in the survival of patients with Burkitt's lymphoma. Data is too preliminary at this point for further comment. We hope to accumulate enough patients to determine whether the morphological distinction of Burkitt's lymphoma is clinically valid.

2. Protocol 78-06 (Relapse Therapy) - The previous high-dose therapy protocol ("BACT") for recurrent lymphoma was closed after treating a total of 23 patients (19 Burkitt's lymphoma). This protocol resulted in 4 disease-free survivors with remission durations of 30, 44, 54, and 64 months to date. Protocol 78-06 is a pilot protocol which attempts to take the best elements of BACT, and add total body irradiation to improve outcome. Autologous marrow infusion is used if available, and if unavailable, TBI will not be a part of therapy. So far, 4 patients without marrow available have been treated with cytoxan (180 mg/kg/total dose) and cytosine arabinoside (2100 mg/M² total dose). One is alive and disease free at 21 months, one died after failure to recover peripheral counts, and 2 died with resistant disease. Four additional patients have been treated with the above regimen plus low dose TBI (total 120 rads) and autologous marrow reinfusion. All patients relapsed at approximately the time of marrow recovery (30-36 days from day 1 of treatment). Two patients received high-dose TBI (800 rads) in addition to cytoxan and cytosine arabinoside. Both died after initial good responses.
3. Protocol 78-09 (Phase II Study of Iphosphamide in Refractory Non-Hodgkin's Lymphomas) - Previous studies have demonstrated that Iphosphamide can induce partial and complete remissions in lymphoma patients resistant to prior cyclophosphamide therapy. Therefore, the present protocol has been instituted to study the activity of

Iphosphamide in juvenile lymphomas refractory to cyclophosphamide. Iphosphamide is given intravenously at a daily dose of 1200 mg/m² for 5 days; constant infusion is utilized. The course of treatment is repeated every 2-3 weeks. Eight patients have been entered on the study. There was one complete response (a patient with diffuse undifferentiated lymphoma who received three courses prior to relapse). One patient with Burkitt's lymphoma had complete regression following the initial course of Iphosphamide, but relapsed prior to the second course. Five patients had partial responses and one no response.

4. Other Observations in Lymphomas - Clinical and laboratory studies in the last year have resulted in significant progress in our understanding of lymphoid malignancies in young people. A tentative hypothesis has been proposed relating B cell lymphomas to the various stages in B lymphocyte differentiation, as characterized by the expression of surface immunoglobulin and complement receptor subtypes. In this context, the relationship between African and American Burkitt's lymphoma is becoming clearer and the validity of the morphological separation of Burkitt's lymphoma from undifferentiated lymphoma in American patients has been called into question. Hopefully, an answer to this question will be provided when current studies are completed. The high frequency of 8:14 chromosome translocations in American BL has been documented, and its significance is being further explored. We have completed further studies of the tumor cell lysis syndrome, documenting marked urinary excretion of phosphate shortly after therapy. It is probable that high urinary phosphate levels and possible precipitation of phosphates in renal tubules contributes to the renal functional defect in this syndrome. Studies of lymphoma cells have documented the major division in young people into B and T types, while studies utilizing fluorescence polarization of the hydrophobic probe DPH have shown interesting disease-specific ranges of whole cell "microviscosity" which appears to relate to the cytoplasmic content of neutral lipid.

H. Ewing's Sarcoma

1. Background: - In October of 1976 patients on the Ewing's Sarcoma study were transferred from the Radiation Oncology Branch to the Pediatric Oncology Branch. A total of 117 patients with histologically documented Ewing's Sarcoma had been entered on a series of Radiation Oncology Branch studies beginning in 1964 and continuing until the transition time. Of these patients, approximately 58 remained as active patients. These patients had been treated with 5000 rads to the primary lesion and with a series of progressively more intensive adjuvant chemotherapy programs. The first of these, denoted S1, consisted of cyclophosphamide given as either a single dose or a series of short courses. The second

consisted of cyclophosphamide + vincristine, again given as a series of short courses. The third regimen consisted of cyclophosphamide, vincristine and actinomycin D as systemic chemotherapy, coupled with 2000 rads of whole brain radiation and a single dose of intrathecal methotrexate. The fourth adjuvant regimen was similar to the third with the exception that adriamycin was substituted for actinomycin D. We have performed a series of studies on the overall group of Ewing's sarcoma patients with the idea of establishing the efficacy and complications of the treatment modalities that have been employed to date in order to take advantage of those aspects of treatment which have been successful and to modify those aspects of treatment which are unsuccessful or unacceptably toxic. We have therefore analyzed and updated the total experience with Ewing's Sarcoma utilizing the treatment protocols employed by the Radiation Oncology Branch during 1964 to 1976. The overall results of these analyses indicate that the treatment groups were relatively unbalanced with respect to prognostic factors at presentation, in particular, site of primary disease and serum lactate dehydrogenase levels at presentation. These imbalances prevent any firm conclusions being drawn as to the overall efficacy of later treatment protocols with respect to earlier results. The results are inconclusive whether disease-free survival or overall survival are taken as the endpoint of the analyses. Analysis of local recurrence rates for the overall group of patients indicates that metastatic status at presentation and adjuvant chemotherapy employed have no effect on local recurrence rates. Local recurrence rates were lowest for patients presenting with distal lesions (40/o), next highest for femoral lesions (120/o), next highest for lesions of the central axis (330/o), and highest for lesions of the humerus (570/o). It is of note that the number of patients presenting with lesions of the humerus was very small (7 patients) and the results are therefore subject to a sizeable error. The analysis was carried out using both anti-mortem and post-mortem results, with recurrence rates analyzed by those recurrences which terminated initial remission, those recurrences which terminated subsequent remissions during the patient's clinical course, and those recurrences which were only detected at post-mortem examination. If the analyses are restricted to clinical recurrence rates only, the results are comparable to previous data obtained by the Intergroup Ewing's Sarcoma Study. When post-mortem data is taken into account, the local recurrence rate increases by approximately one-third, primarily in patients presenting with central lesions. In this study, in contrast to the results previously mentioned, serum lactate dehydrogenase level at presentation was not a prognostic indicator. It is also of note that both pelvic and non-pelvic central axis lesions had similar rates of local recurrence. The results of this analysis indicate that 5000 Rad whole bone irradiation is satisfactory treatment, both in terms of local

control and overall therapeutic results, for lesions below the elbow or below the knee, and lesions of the femur. Local recurrence rates are higher for lesions of the central axis and for lesions of the humerus, indicating that modification of treatment for these groups of patients is indicated. Functional results for patients with lower extremity lesions were evaluated in 22 patients. Patients presenting with lesions of the upper extremity were not evaluated because there were not sufficient numbers. The minimum follow-up period for entry into this study was three years. Results indicate that only 1 of the 22 patients required amputation for morbidity associated with treatment. The majority of patients had highly acceptable functional results. We conclude, therefore, that radiation therapy is satisfactory treatment for lesions of the extremities and that amputation should not be considered because of the possible toxicities of radiation therapy. Patients presenting with metastatic lesions, irrespective of the site of primary disease, did very poorly with any of the Radiation Branch protocols, with no median disease-free survival interval, and a median survival interval of 22 months. The maximum disease-free survival interval for patients presenting with metastatic disease on the Radiation Branch studies was 70 weeks (Protocol S4, which consisted of radiation therapy to the primary lesion plus 4 cycles of vincristine, cytoxan and adriamycin).

2. New Studies (77-05, 06, and 07) - With the background outlined above and with the availability of intensive supportive care measures in the Pediatric Oncology Branch, new protocols were begun in 1976 for patients presenting with metastatic and pelvic disease, patients presenting with disease of the extremities without metastases, and patients who relapse on prior protocols. Patients presenting with pelvic or metastatic disease are treated with combination chemotherapy plus radiation to the primary site and all known metastatic sites, plus low-dose (150 rads) total body irradiation followed by intensive high-dose chemotherapy and autologous marrow infusion. Patients presenting with lesions of the extremities are treated with combination chemotherapy, radiation to the primary lesion, pulmonary irradiation, and maintenance chemotherapy. Patients who relapse from prior protocols are treated with a regimen that is similar to that used for patients presenting with pelvic or metastatic disease. Results to the present time suggests that for patients presenting with metastatic disease, this regimen yields significantly better results than any prior protocol used at the National Cancer Institute. This result also suggests that total body irradiation can act as a systemic agent in combination with chemotherapy to improve therapeutic results in patients with Ewing's sarcoma. Since these results have achieved significance at the $p=.001$ level, we are presently planning a new protocol for these patients which will build on the results of the present group of protocols,

utilizing a different fractionation of the total body irradiation and a different approach to treatment of the primary lesion. Results in patients presenting with lesions of the extremities are as good as, but not statistically superior to, results of prior protocols at the National Cancer Institute. The number of patients entered on this protocol makes a large statistical difference between the present results and prior results unlikely. We are therefore planning to follow the present group of patients further in time more adequately to assess these results, to redesign the protocols to take better advantage of the improved understanding that has been gained by the present studies, and to initiate a new study which will be a more radical departure from the previous study. We are also presently examining patterns of relapse in patients with Ewing's sarcoma to evaluate the present approach to relapsed patients and to modify this approach accordingly.

3. Ancillary Studies: - We have completed a study of the incidence of second malignancies in patients with Ewing's sarcoma treated at the National Cancer Institute and compared these results with a similar study that was performed at the M. D. Anderson Hospital. Thirty-one patients were evaluated. Of these 31 patients, 2 patients developed second malignancies, 1 patient developed a radiation-induced fibrosarcoma, and another a neuroblastoma outside the radiation field. No patient developed osteosarcoma. These results are different from the results at the M. D. Anderson Hospital where 4 patients, out of 24 long-term survivors, developed osteosarcoma. In collaboration with the Epidemiology Branch, we are also evaluating the incidence of genito-urinary anomalies in patients presenting with Ewing's Sarcoma, and in families of such patients. The results to date indicate that 5 of the patients in this series have genito-urinary anomalies. This represents an observed incidence of approximately 5 times the expected incidence of such anomalies. The remainder of the group of patients with Ewing's Sarcoma is undergoing analysis at the present time. Initial attempts are being made in collaboration with the Laboratory of Molecular Pharmacology to evaluate the ability of patients with Ewing's Sarcoma and family members of patients with Ewing's Sarcoma to repair alkylating-induced damage of DNA in peripheral lymphocytes, utilizing a fluorescent assay that was reported by Erickson and collaborators.

I. Miscellaneous Phase I and II Studies:

1. Dihydroxyanthracenedione and ICRF 187 - We are presently engaged in two Phase I trials, dihydroxyanthracenedione and ICRF 187. Both of these trials have already reached drug dosage levels which are higher than the maximally tolerated doses in adults, and are continuing at the present time. The ICRF 187 dose is at 2500

mg/m²/d x 3, and the dihydroxyanthracenedione dose is at 16 mg/m² every 28 days. Adult studies established a maximal tolerated dose of 1250 mg/m² for ICRF on a 3 day schedule and a maximally tolerated dose of 12/mg/m² on a once/28 day schedule for dihydroxyanthracenedione. The implication of these studies is that pediatric patients tolerate higher doses of these agents than do adults, and that new anti-cancer drugs should be evaluated independently in children.

2. Relapse ALL: Phase I study of 2'-Deoxycoformycin - As part of an evaluation of promising new agents in relapsed patients with ALL, we recently conducted a Phase I study of 2'-deoxycoformycin in a cooperative effort with two other institutions (St. Jude Children's Research Hospital and the Sidney Farber Cancer Research Institute). This agent (2'-dCF) is a known inhibitor of the enzyme adenosine deaminase (ADA). ADA is an enzyme of known importance in lymphocyte metabolism. It is present in highest amounts in lymphoid tissues and absence of the enzyme, as occurs with genetic absence of ADA, results in defective lymphocyte maturation and function (i.e., severe combined immunodeficiency disease). Our interest in 2'-dCF as an anti-leukemia agent was stimulated by our observation that the leukemic blast cells of ALL patients with "T Cell disease" have considerably higher ADA levels than "Non-T, Non-B" lymphoblasts, prompting speculation that, should it be effective against leukemia, 2'-dCF may have "specificity" for poor prognosis patients with T lymphoblasts. Thus far, 26 patients with refractory ALL have been treated with 2'-dCF. Ten of these patients had T-cell disease, the remainder had non-T, non-B cell leukemias. The dose initially utilized for the study was 0.25 mg/kg iv for 3 days. It was subsequently increased to 0.5 mg/kg and then to 1 mg/kg. Mild toxicities noted with the drug include nausea, vomiting, diarrhea and conjunctivitis. Dose limiting toxicity includes effects on the central nervous system (confusion, seizures, encephalopathy), the kidney (acute renal failure), and the liver. The 0.5 mg/kg dose has been associated with only mild toxicity and has been demonstrated to inhibit the adenosine deaminase of lymphoblasts. Of the 26 patients treated to date, 2 have achieved complete remission and partial responses were noted in 4 individuals. The results of this study suggest that the .50 mg/kg dose, on a qd x 3 iv schedule, is a safe dose for future studies with this agent. In addition, this study confirms the activity of 2'-Deoxycoformycin in acute lymphoblastic leukemia.
3. Phase II Trials of Polyriboinosinic-Polyribocytidylic Acid Stabilized with Poly-L-lysine, in Carboxymethylcellulose [Poly(ICLC)], a Highly Effective Interferon Inducer - Poly(ICLC) resists hydrolysis by primate serum (unlike the parent compound), induces high levels of serum interferon, and is effective in acute viral infections of subhuman primates. In a Phase I clinical

trial, toxic reactions included fever (in 100% of trials), nausea (44%), hypotension (28%), thrombocytopenia and leukopenia (68%), erythema (12%), and polyarthralgia plus myalgia (16%). Hypotension and arthralgia-myalgia related to dose level and/or magnitude of interferon induction, but other toxic manifestations did not. Poly(ICLC) induced significant serum interferon levels in 76% of trials, and the correlation between dose and peak interferon titer was linear. The maximum tolerated dose for all patients at a given drug dose was 12 mg/sq m; at this dose, the mean peak interferon titer was 1940 reference units/ml. We have undertaken several Phase II trials of poly(ICLC) at doses up to the MTD of 12 mg/sq M. In 16 NCI patients with AML, Burkitt's lymphoma, osteosarcoma, or Hodgkin's disease, there have been no responses. Among 18 patients with refractory ALL studied by the CCSG, there has been one initial response, but the patient died before the response could be fully evaluated. Five patients have been studied at the University of Arizona with multiple myeloma; all 5 have experienced a decrease in their plasma or urine M-component. Finally, 2 children at Johns Hopkins with juvenile laryngeal papillomatosis have required less frequent surgery while receiving poly(ICLC). These results suggest that certain tumors may show a biological effect of interferon inducers, as they appear to do with interferon per se; the ultimate clinical significance of this effect remains unproven. Moreover, our observation that poly(ICLC), at a tolerable dose, is the first consistent inducer of high serum interferon levels in humans, suggests that it should be studied in certain human viral infections.

J. Parenteral Hyperalimentation as an Adjunct to Intensive Treatment

Malnutrition is a frequent complication of childhood cancer and/or its treatment, and contributes to the loss of integrity of the integumentary and mucosal barriers, impairs phagocytosis, decreases macrophage mobilization, and depresses lymphocyte function which leads to infection. In a randomized clinical trial in patients receiving intensive (i.e., "ablative" chemotherapy) we seek answers to the following questions: 1. Does the assurance of optimal nutrition via parenteral alimentation improve the tolerance of the patient with cancer to aggressive chemotherapy? 2. What influence does nutritional support have in the response of the tumor to aggressive chemotherapy, i.e., can a clear difference in patient survival be demonstrated? 3. Are "host defenses", as measured by *in vivo* parameters of immune response, improved by maintenance of optimal nutrition? 4. Are the management problems of these patients made more difficult as a result of their total parenteral nutrition? In this trial, the patient receives total parenteral nutrition of 1500 - 4000 calories per day (40 calories per kg with 0.30 gm of nitrogen per kg ideal weight) as a mixture of 25% Dextrose and 4.25% Freamine. Total parenteral nutrition continues until oral intake is established at >2000 cal/day.

To date, 25 patients have been entered onto this protocol: 16 as controls and 9 randomized to receive TPN. Several preliminary inferences can be drawn from these trials: First, even in patients with protracted granulocytopenia and severe immunosuppression, central lines can be maintained without infectious sequelae if monitoring and guidelines for use are strictly adhered to. Second, 75% of patients undergoing intensive chemotherapy who did not receive concomitant hyperalimentation experienced weight loss (mean 3.1%, range 1-11%) while 8/9 patients receiving hyperalimentation had a weight gain during the chemotherapy and post-chemotherapy recovery period (mean 2.7%, range 1-6%). This short term benefit in weight preservation is associated with fewer deaths during therapy (3 for nonalimentated, 1 for hyperalimented), but the time to recovery and the long-term efficacy of chemotherapy was not enhanced by the hyperalimentation regimen. Metabolic and immunologic studies of these patients are in progress. At this juncture, hyperalimentation does not appear to significantly alter the long-term benefits of chemotherapy, although further study is warranted.

K. Psycho-social Concomitants of Cancer

We have previously reported on the families of patients who have life-threatening illness and on physicians who care for such patients. In particular, we explored the factors in the doctor-patient relation which promote anxiety in the doctor to the end that he is less effective than he might be. During the past year, our annual seminar for Clinical Associates on the Doctor-Patient Relation has continued with Dr. Harold Greenberg as the seminar leader. The seminar continues to be highly successful and has proven to be a model for many other institutions throughout the country, including cancer centers and general hospitals. We have also continued a collaborative research effort with the Laboratory of Developmental Psychology, NIMH (M. Yarrow, Chief). The objective of this research is to analyze developmental stages in young patients who have chronic and/or life-threatening disease, to learn the factors which promote normal or abnormal development, and the manipulations which may be of value in dealing with developmental abnormalities. We are continuing studies on the nature of communication between staff, family and patient; the effects of isolation within the laminar-flow room as a function of the age of the patient; and the effects on mentation in patients who have received prophylactic irradiation of the central nervous system (as noted elsewhere, we have detected a significant drop in I.Q. among patients so treated).

Significance to Biomedical Research and the Program of the Institute:

Significant progress has been made in delineating the cell of origin in acute leukemia and in identifying factors which may be useful in predicting response to therapy. By further defining the acute leukemias vis-a-vis

these prognostic factors, we hope to refine therapy to the end that remission rate and duration will further improve. In this Branch 430/o of patients with ALL entered in our most recently completed study are free of disease after having been off all treatment for 3 - 6 years. We have learned that the acute leukemia cell has extraordinary antigenic and biochemical complexity: the effects of both specific and nonspecific immunotherapy in this disease have been discouraging, but our understanding of immunological and biochemical mechanisms involved in leukemia has increased. In this regard, we have identified a new agent (2'-deoxycyformycin, an inhibitor of lymphocyte adenosine deaminase) which may be effective in relapsed acute lymphocytic leukemia.

The central nervous system is a major site for relapse in acute leukemia. Our studies on route and scheduling of CNS chemotherapy promise an effective and relatively nontoxic approach to this important problem. It is further evident that the most important contribution that can now be made to the treatment of "good risk" patients with acute lymphocytic leukemia is to provide effective CNS prophylaxis without the significant toxicity we have detected in association with cranial irradiation (CT scan abnormalities; neurologic, intellectual, and psychologic dysfunction; growth hormone deficits). By exploiting the elegant monkey model for CNS pharmacokinetics developed in this Branch, the very sophisticated monitoring techniques available to us (e.g., C.T. scans), and our experience in clinical pharmacokinetics, we hope to develop an effective CNS prophylaxis to be administered systemically and without significant sequelae.

We are beginning to make progress in the treatment of advanced solid tumors that have been refractory to conventional doses of combination chemotherapy. The response in non-Hodgkin's lymphoma has been particularly gratifying in that we now believe that if successful CNS prophylaxis can be developed, we will achieve durable remissions in about 800/o of patients, including those presenting with extensive disease. We further believe that the concept of high-dose combination chemotherapy together with maximal supportive care (including, for example, autologous marrow rescue) will be useful in metastatic osteogenic sarcoma (after metastatectomy), rhabdomyosarcoma, and Ewing's sarcoma, although our evidence is disappointing in the case of metastatic neuroblastoma. Early intensive treatment of newly diagnosed patients with high-risk (but low bulk) disease may be of particular value (as hypothesized in the mathematical model of Norton and Simon, wherein the low growth rates of small tumor volumes necessitate high dosages of chemotherapy for effective cyto-eradication). While such aggressive therapy is not without toxicity, our experience to date suggests that it can be successfully delivered, and that it may be of benefit to such poor prognosis patients. In each of the solid tumors we are exploring other interesting observations: the synergistic possibilities of treatment with cis-platinum and adriamycin in the case of metastatic osteogenic sarcoma, pharmacological debulking in the case of localized rhabdomyosarcoma, and the suggestive efficacy of total body

irradiation and prophylactic pulmonary irradiation in the case of Ewing's sarcoma. We have made considerable progress in the development of multi-resource approaches to the psycho-social concomitants of cancer in patients, their families, and therapists. We believe that this approach is exceedingly important, for failure to deal with these concomitants may be reflected in inadequate treatment of cancer. We believe that the Pediatric Oncology Branch has provided a model for coordinated research and care in the psycho-social concomitants of chronic illness which has proven applicable to many other diseases and institutions.

Finally, it should be noted that certain of our studies relate to clinical concerns that are not restricted to cancer treatment: For example, our clinical trials of poly(ICLC) have for the first time demonstrated consistent and significant interferon induction with tolerable toxicity in man. These data should prove useful in a wide variety of infectious disease problems, e.g., control of the hepatitis B carrier state.

Proposed Course:

All of the studies enumerated previously will be continued. Aggressive protocols have been developed for acute leukemia patients who relapse from primary induction protocols but remain sensitive to extant chemotherapy. Increased emphasis will be given to phase I-II trials, in particular studies of poly I-poly C/poly-l-lysine, dihydroxyanthracenedione, deoxycoformycin, l-phosphamide, and ICRF-187. We shall continue to explore the pharmacokinetics of methotrexate so as to improve the therapeutic index of this drug as it is used in the treatment and prophylaxis of meningeal leukemia. Experiments on the use of high-dose chemotherapy in refractory and metastatic solid tumors will be expanded; in these studies, chemotherapy is assessed in a situation whereby maximum supportive care is possible, including autologous bone marrow rescue. Our interest in laminar-flow-room prophylaxis, granulocyte transfusions, total parenteral nutrition, and empiric antibiotics will continue *pari passu* with studies of high-dose chemotherapy. We plan to expand studies in clinical and molecular pharmacology and to utilize the results of such studies in planning further clinical chemotherapy trials. Both our clinical and basic studies in the kinetics of hematopoietic reconstitution and differentiation will be used to guide further experiments on clinical marrow reconstitution, using cryopreserved marrow (or stem cells obtainable from the peripheral blood). In collaboration with the Laboratory of Developmental Psychology, NIMH, we plan to expand our study of stages in normal and abnormal psychological development as they relate to the setting of chronic illness. Finally, we shall expand our interest in the etiology of cancer in the young with further studies of the 8:14 translocation in Burkitt's lymphoma, repair of alkylation injury in Ewing's sarcoma, and aryl-hydrocarbon hydroxylase inducibility in leukemia and solid tumors.

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PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Hematological Support of Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
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☒ (a) HUMAN SUBJECTS☒ (b) HUMAN TISSUES☐ (c) NEITHER☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Hematopoietic reconstitution using autologous marrow cells is being evaluated as an adjunct to trials of intensive therapy in resistant sarcomas, small cell carcinoma of the lung, and undifferentiated lymphoma. Pharmacologic amplification of peripheral blood levels of autologous stem cells is being studied as a means of accelerating both immunological and myeloid recovery following intensive therapy in man and in a dog model. Studies of the interaction of autologous peripheral blood and marrow stem cell infusions during reconstitution is being conducted in an animal model. Studies in vitro and clinical trials are undertaken to define the optimal platelet support of cancer patients. Extracorporeal hemoperfusion through immunoadsorbent columns, using continuous flow centrifugation in a canine model and in clinical trials, is being studied as a means of inducing regression in tumors resistant to established forms of therapy. Finally, extensive in vitro studies of changes in immune complexes, suppressor cell activity, and various arms of the cellular immune response are being pursued in an attempt to elucidate the mechanism of tumor regression seen following immunoadsorbent therapy.

Objectives:

1. To reconstitute patients with autologous hematopoietic stem cells following intensive chemotherapy and/or irradiation of Ewing's sarcoma, small cell carcinoma of the lung, soft tissue sarcomas and undifferentiated lymphomas, using stem cells collected from marrow or peripheral blood of man; to amplify the numbers of peripheral blood hematopoietic stem cells so as to permit collection of an adequate number of such cells during a single continuous flow centrifuge collection; to study the biology of hematopoietic reconstitution using animal models as well as model systems in man (e.g., post-ablation recovery); to characterize the interaction of marrow and peripheral blood in accelerating immune and myeloid recovery following intensive therapy; and to extend our data on the dose of CFUc/kg needed for engraftment in the animal model to man using both autologous peripheral blood and marrow cells. We are also studying the efficacy of autologous cryopreserved marrow cells to increase tolerance to subsequent maintenance chemotherapy.
2. The preclinical canine model will be used to determine if the amplification of CFUc (committed myeloid precursor cells), which we have observed in patients during hematopoietic recovery from conventional dose combination chemotherapy, also occurs in dogs during hematopoietic recovery from chemotherapy. If we are able to reproduce this amplification phenomenon in dogs, we will then collect varying numbers of mononuclear cells from the peripheral blood of animals containing amplified CFUc peripheral blood levels, and test these preparations for their ability to completely reconstitute animals following total body irradiation. If we find that the minimal dose of peripheral blood mononuclear cells required to reconstitute animals following total body irradiation is lower in collections made from animals with amplified populations of peripheral blood mononuclear cells, this result will imply that such preparations contain amplified levels of pluripotent stem cells. We will use this information to develop means of collecting adequate numbers of peripheral blood mononuclear cells from patients who require autologous stem cell reconstitution but whose autologous marrow cells cannot be collected.
3. To extend the encouraging results of the capillary platelet migration inhibition test and the results of our retrospective analysis of determinants of good post-transfusion platelet increments in alloimmunized recipients. We wish to identify in a clinical trial which cancer patients will profit from the transfusion of HLA-matched platelets as opposed to single-donor unmatched platelet transfusions. To assess the incidence of HLA antibodies in these patients compared to aplastic anemia patients.
4. To assess the continuous flow centrifuge as a means of supportive treatment for a variety of diseases. Neocyte collection (young red cells) is being used to decrease the transfusion requirements of

patients with aplastic anemia or other disorders associated with transfusion hemosiderosis (e.g., thalassemia). Lymphocyte depletion is being studied for its possible effect on rheumatoid arthritis.

5. The canine model is being used to evaluate critically the recent observation that passage of the animal's blood over staph A antigen (on nitrocellulose filters connected to a continuous flow centrifuge plasma line) will produce lysis of various canine neoplasms. Responders are being compared to non-responders with respect to changes in immune complexes as they may relate to predictive indices of response and to the mechanism of tumor regression. In addition, clinical trials (phase I and phase II) are being carried out in man using this immunoadsorbent technique. A battery of in vitro tests of both humoral and cellular immune response is being employed to attempt to elucidate the mechanism of the tumor regressions seen to date using this procedure. Finally, eluates from these immunoadsorbent filters are being studied with the hope of developing preparations of antitumor antibodies and tumor specific antigens for use in biological studies as well as in diagnosis.

Methods Employed:

In vitro assays are used to identify the numbers of stem cells of erythroid or myeloid origin present in marrow and peripheral blood samples. These assays involve incubation of nucleated hematopoietic cells in semisolid culture media (methyl cellulose or agar). The number of colonies formed in 10-14 days constitutes an indirect measure of the stem cell content. Automated temperature-controlled instruments are used for the cryopreservation of marrow. Aminco continuous-flow centrifuges, Haemonetics discontinuous-flow centrifuges, and the Fenwal filtration leukapheresis device are used for the collection of granulocytes and platelets, as well as stem cells, neocytes and lymphocytes. This latter component is also collected by conventional non-automated centrifugational methods ("split bag method"). Collection of mononuclear cells from the peripheral blood is accomplished with the Aminco continuous-flow centrifuge. An intramural laboratory equipped with advanced culture and analytical techniques (cytofluorograph, scintillation spectrometers, ultracentrifuges, laminar flow hoods, phase bright-field and fluorescent microscopes) is supported by contract facilities in which canine experiments, in vitro assays of platelet compatibility, and histo-compatibility phenotyping are conducted. A donor file of 20,000 individuals (all of whom are typed with respect to the A and B loci of the HLA system) is backed up by an interlocking network of 13 centers for donor procurement. Finally, a serum repository with at least 14,000 entries is maintained for clinical and laboratory investigations. A battery of granulocyte functional assays is available (bactericidal capability, phagocytosis, chemotaxis, histological and biochemical assays for enzymes specific for mature neutrophils, and assays for substances within the specific and non-specific granules of mature myeloid cells). In vitro tests for immune complex levels in dogs and man have been developed.

Major Findings:A. Hematopoietic Recovery with Marrow Infusion Following Intensive Therapy

1. Cryopreserved Marrow Studies - Previous studies have demonstrated that autologous hematopoietic stem cell infusions can avert the otherwise irreversible myelosuppression which would follow regimens employing 800 rad or more of total body irradiation (TBI) in combination with high dose Cyclophosphamide or other chemotherapeutic agents. However, the efficacy of autologous stem cell infusions in accelerating hematopoietic recovery following regimens of an intermediate level of intensity is as yet unresolved. We therefore embarked upon a study of the ability of frozen cryopreserved autologous marrow to reverse the myelosuppression which follows combined modality therapy. Hematopoietic recovery data in patients receiving suboptimally frozen marrow infusion, and in patients not receiving marrow infusion, have been pooled for purposes of analysis. We chose to approach the data in this way because of previous studies showing decreased cell survival following excessive rates of freezing and our own *in vitro* data on CFUc numbers before and after optimal and suboptimal rates of freezing. These studies, performed on marrow preparations obtained from bone marrow storage of five patients, indicate that rates of cooling during cryopreservation which exceed $6^{\circ}\text{C}/\text{min}$ are associated with post-freeze CFUc survival of 0-11% (n=3). In contrast, marrow preparations frozen at cooling rates less than $6^{\circ}\text{C}/\text{min}$ throughout the entire procedure are associated with post-freeze CFUc survival between 50 and 70% (n=5). The median time to recovery of the total white blood cell count to greater than $1000/\text{mm}^3$ was 27 days in patients receiving adequately frozen marrow and 45 days in the remaining patients ($p < 0.001$). Similarly, the median time to platelet recovery (to greater than $50,000/\text{mm}^3$ unsupported by platelet concentrate transfusion) was 4.5 weeks as compared to greater than 11 weeks, and the median time to granulocyte recovery (to greater than $500/\text{mm}^3$) was 29 days as compared to 55 days ($p < 0.001$ in both cases). These data demonstrate that in the nonlethal but intensive treatment setting, infusions of adequately cryopreserved autologous hematopoietic stem cells accelerated recovery of granulocyte counts to a level of $500/\text{mm}^3$ by 3-4 weeks.
2. Dose of Nucleated Cells and CFUc Leading to Hematopoietic Reconstitution Following 900 Rad of Total Body Irradiation, Using Bone Marrow Cells and Peripheral Blood Cells - In order to establish the dose of colony forming units (committed myeloid precursor cells) which is associated with complete hematopoietic reconstitution following 1000 rad of total body irradiation, marrow cells were stored from dogs as described above and cryopreserved after measuring the content of total nucleated marrow cells and total CFUc/ 10^6 mononuclear cells. Different doses of total

mononuclear cells were stored from various dogs and the number of CFUc in each preparation was measured. Following treatment of each animal with 900 rad of total body irradiation, each of these preparations containing different doses of total marrow nucleated cells and CFUc was infused into each animal, permitting us to test the minimum dose of each associated with complete hematopoietic recovery following total body irradiation. These studies demonstrated that 0.5×10^8 nucleated marrow cells were adequate for complete hematopoietic recovery, as shown by other workers, and that 4×10^3 CFUc/kg were adequate for complete hematopoietic reconstitution using marrow or peripheral blood.

3. Use of Peripheral Blood Mononuclear Cells for Hematopoietic Reconstitution in Man - We attempted syngeneic hematopoietic reconstitution using peripheral blood mononuclear cells from the normal identical twin of a patient with Ewing's sarcoma. This patient's bony pelvis was extensively compromised by tumor and adequate bone marrow storage could not be effected. The existence of an on-going protocol involving intensive treatment (150 rad fractionated TBI and high-dose chemotherapy) for patients with high risk or metastatic Ewing's sarcoma provided us with data on a cohort of similarly treated patients managed with and without hematopoietic reconstitution against whom comparison of a peripheral blood reconstitution could be made. The normal identical twin underwent a pheresis regimen that yielded 8.7×10^{10} mononuclear cells (containing 4×10^5 CFUc) from 8 separate pheresis procedures. The collections of these cells represented approximately 9 times the number of cells usually obtained from a bone marrow storage, but only 1/8 the number of hematopoietic stem cells (as judged by CFUc measurement) normally found in a bone marrow collection. This infusion eventuated in accelerated lymphocyte recovery comparable to that seen following hematopoietic reconstitution with autologous marrow, but granulocyte and platelet recovery were not accelerated. After 7 weeks of observation the study was terminated by the infusion of syngeneic bone marrow cells from the normal identical twin, resulting in prompt hematopoietic recovery over the following 14-21 days. We attributed this limited result to the paucity of hematopoietic stem cells we were able to find in these collections.
4. Amplification of CFUc in Peripheral Blood of Man - Prompted by reports of expanded numbers of circulating hematopoietic stem cell following cyclophosphamide treatment in mice, and conflicting reports regarding CFUc behavior following chemotherapy in patients, we elected to study the behavior of circulating CFUc numbers in patients undergoing intensive induction therapy for extensive small cell carcinoma of the lung. Over a 14-month period we were able to study 20 such patients and observed an expansion of 6.9 fold in circulating CFUc (range 0.9 to 27 fold) with 90% of patients demonstrating at least a two-fold expansion. The value of this

observation was enhanced by the concomitant observation that maximal expression occurred during leukocyte recovery as measured by routine blood counts. Since CFUc measurement requires 10-14 days to complete, the ability to predict CFUc behavior indirectly through leukocyte behavior is of great value. We calculated that 2 collections of peripheral blood mononuclear cells, each containing 1×10^{10} cells, collected during CFUc expansion, would yield CFUc numbers comparable to the numbers found in a bone marrow collection obtained for reconstitution purposes.

5. Peripheral Blood Stem Cell Amplification Following Chemotherapy in a Dog Model - In order to evaluate the reconstitutive significance of chemotherapy-induced CFUc expansion, we turned our attention to our preclinical canine model for hematopoietic reconstitution. In particular, we wished to know whether expanded CFUc numbers would equate with increased reconstitutive potency per mononuclear cell and whether cells collected acutely following chemotherapy would still be effective for reconstitutive purposes. The following table shows the scheme of this study:

Table. Study of Peripheral Blood Stem Cell Amplification in Dogs

1. Cytoxan, 16 mg/kg on days 0 and 7
2. Collection of peripheral blood mononuclear cells day 15-16
3. Cryopreservation of buffy coat preparations
4. Recovery
5. Total body irradiation, 900 rad
6. Conventional hematological support
7. Reconstitution with varying doses of mononuclear cells and CFUc

A series of dogs treated in the manner outlined above were subjected to collections on the continuous flow centrifuge. Preparations collected from these animals during the period of CFUc amplification were characterized with respect to their content of CFUc and total mononuclear cells. Preparations with different numbers of total peripheral blood mononuclear cells were frozen and then reinfused into the same dogs following treatment of these dogs with 900 rads of total body irradiation. We then measured the capability of each of these preparations to effect complete hematopoietic reconstitution. The results of these studies showed that the minimum number of peripheral blood mononuclear cells needed for complete hematopoietic reconstitution following total body irradiation was reduced by 10- to 20-fold when the preparations were obtained from animals at the time of cytoxan-induced CFUc amplification. Thus, these data indicate that amplification of CFUc cells in these preparations had increased reconstitutive capability. The required number of stem cells can be obtained from the peripheral blood if amplification of the normal levels of pluripotent stem cells has taken place, using a minimum of two 4-hour continuous flow centrifugation runs.

B. Regression of Mammary Carcinoma Following Extracorporeal Immunoadsorbent Therapy

The existence of an immunologic response to tumors has been documented by several studies. However, this response may be rendered ineffective by factors in the circulation which appear to have the ability to block the cytotoxic mechanisms directed against tumor cells. In vitro studies have shown that cell-mediated cytotoxicity (CMC) may be ineffective in the presence of sera obtained from the host, but active in "normal" sera, thus implying the presence of soluble immunosuppressive substances(s). These "blocking" factors seem to develop in the early stages of tumor growth, and have been shown to be present in sera of animals and humans with growing tumors, but absent following extirpation of those tumors. Furthermore, in animal studies, transfer of "blocking sera" has enhanced growth of transplanted tumors. Removal of blocking activity with resultant restoration of CMC has been variously accomplished by washing of host lymphocytes, filtration of host sera, use of "unblocking" sera, and plasma exchange. Clinical improvement in humans has been reported following the use of plasma exchange and extracorporeal immunoadsorption.

Protein A, found on the cell wall of Cowan I strain *S. aureus*, has the characteristic of binding IgG via the Fc fragment of the immunoglobulin molecule. In addition, it has been shown that IgG complexed with antigen binds more readily than, and is not easily displaced by, free IgG. Free antibody and antigen-antibody complexes may inhibit CMC, and both have been demonstrated in sera from human tumor hosts. In a clinical investigation, perfusion of plasma through a filter prepared with Cowan I strain *S. aureus* has been reported to result in significant tumor reduction in a patient with colon carcinoma. A clinical study of the effect of extracorporeal perfusion through a staph A column for the treatment of lymphocytic lymphoma has also demonstrated regression. This same technique has been applied to the treatment of canine mammary tumors with beneficial results (Terman, D., unpublished data). Accordingly, we have begun an investigation of the effects of extracorporeal immunoadsorption in spontaneous canine tumors. The use of inactivated *S. aureus* would provide a mechanism for nonspecifically removing circulating immunosuppressive complexes and free antibody. Four of 9 dogs tested exhibited tumor regressions following treatment, whereas none of 4 dogs treated with perfusion of blood through filters without *S. aureus* exhibited a response. Tumor biopsy pre-treatment revealed adenocarcinomas, primarily intraductal in location; examination of the excisional post-treatment biopsy specimens revealed a significant infiltration with inflammatory cells, primarily mononuclear, but with a moderate number of neutrophils as well. In addition, marked cellular necrosis had occurred, with degeneration and "ballooning" of cells easily visible throughout the specimen. Viable tumor cells appeared to remain in some specimens.

C. Platelet Transfusion Studies

1. Frequency of anti-HLA antibodies in patients afflicted with leukemia, solid tumors and aplastic anemia - Thrombocytopenia is a frequent accompaniment of intensive chemotherapy for malignant disease; however, hemorrhagic complications have been much reduced by liberal transfusion of platelets. Studies in patients receiving repeated administration of platelets (usually for therapy of primary bone marrow failure) have indicated that alloimmunization often occurs, and this phenomenon is positively correlated with refractoriness to transfusion. HLA-matching of platelet donor and recipient has therefore been utilized in an attempt to improve platelet survival and preserve the efficacy of transfusion. We retrospectively examined both the transfusion records and HLA-antibody screens (by lymphocytotoxicity studies, Dr. P. Terasaki), of the following groups of patients: (1) All patients treated by the P.O.B. for acute lymphocytic leukemia since 1968; (2) patients with Ewing's sarcoma treated with the current P.O.B. protocol; and (3) patients with childhood non-Hodgkin's lymphoma treated with the P.O.B. "BACT" protocol (BCNU, Ara-C, cyclophosphamide, and 6-thioguanine). These groups were chosen because of the intensive nature of the chemotherapy administered, and the consequent high rate of thrombocytopenia and platelet transfusion.

At the present time we have nearly completed the analysis of the leukemia group. The records of 101 patients were examined, and 75 of these were found to be evaluable; the remaining 26 were not considered for analysis due to factors such as lack of sufficient HLA-antibody screens, excessive intervals between platelet transfusion and screening, etc. Forty-four of these patients (61%) never developed HLA-antibodies; this group received a mean ($\bar{x}$) of 74 units of platelets over an interval of 214 days ($\bar{x}$). Ten patients (14%) developed antibodies after administration of a mean of 41 units over an interval ($\bar{x}$) of 105 days. They remained positive only for ($\bar{x}$) 59 days, and reverted to a negative antibody status. They remained antibody negative despite receiving a mean of 146 additional units of platelets over ($\bar{x}$) 303 days. Only ten patients became HLA-antibody positive and remained so; this alloimmunization occurred after having received a mean of 119 units over ($\bar{x}$) 499 days. Conversion to antibody positivity occurred after transfusion of as few as 10 or as many as 321 units of platelets. In addition, seven patients developed HLA-antibodies after transfusion of only packed red cells ($\bar{x}$ of 5 units RBC over an interval of 116 days). Statistical analysis by t-test revealed no significant differences between the amount of platelets given to patients who remained antibody negative, and those who became alloimmunized. Preliminary data, then, indicate that more than 60% of these patients do not become

alloimmunized despite fairly large quantities of platelets given over an average of seven months of treatment. Of the 280/o who did develop anti-HLA antibodies, half reverted to negativity within two months and remained so despite continued administration of platelets over an average period of ten months.

2. Clinical Trial to Evaluate Utility of HLA Matched Versus Unmatched Platelet Transfusions - From statistical analysis, it is estimated that 106 patients are required for this study to be interpretable. At this time, 59 patients have been entered onto the protocol, 21 of which have required platelet support. Only 7 of the 21 have passed to the crossover between phases I and II. The average number of transfusions required by the 7/21 patients who have become refractory to matched or unmatched platelets was 7.8, while those 14/21 patients not refractory to the matched or unmatched arms have required 8.7 platelet transfusions on the average. Thus, most patients entered onto the trial (38/59) have not required platelets yet. Of the patients requiring platelets, most remain responsive to either matched or unmatched (14/21). There is no difference between the number of patients crossing over from the matched or unmatched arms, but the numbers are very small at this time. Also, the anti-HLA, platelet migration inhibition, and lymphocytotoxicity tests are all negative for evidence of alloimmunization. Although more patients are needed for definitive analysis, it appears that no benefit is derived from the use of HLA matched platelet transfusions in a cancer patient population which contains very few patients with AML.

D. Lethal Pulmonary Reactions Associated with the Combined Use of Amphotericin B and Leukocyte Transfusions

Amphotericin B is used increasingly in broad spectrum antibiotic coverage of cancer patients with profound granulocytopenia and suspected sepsis. Recently we observed severe pulmonary reactions associated with Amphotericin in 2 patients who were receiving white blood cell treatment. These reactions, lethal in one patient, were characterized by the sudden appearance of dyspnea, hypoxemia and diffuse interstitial infiltrates during Amphotericin infusion. These cases led us to review the last 6 years' experience with white blood cell treatment at N.I.H to determine if the combination of Amphotericin and white blood cell treatment is associated with pulmonary toxicity not characteristic of either Amphotericin or white blood cell treatment alone. All white blood cell treatments employed cells obtained by centrifugation and were given for granulocytopenia and documented gram negative septicemia. Amphotericin was used during 22 of 57 white blood cell treatment courses. Respiratory deterioration occurred during 14 (630/o) of these courses but in only 2 (50/o) of white blood cell treatment courses without Amphotericin ($p < 0.01$, χ^2). While this difference may reflect selection artifact,

other findings suggested that the combined use of Amphotericin and white blood cell treatment led to respiratory deterioration. In seven cases respiratory function deteriorated acutely during or immediately after Amphotericin infusion, precipitating death in five. Diffuse intraalveolar hemorrhage was found in all cases of acute respiratory deterioration when lung tissue was obtained by biopsy or at autopsy. During the same time period no instances of acute respiratory deterioration occurred in 25 comparably granulocytopenic patients treated with Amphotericin but not white blood cell treatment. Of 22 patients given Amphotericin and white blood cell treatment, the treatments were begun before Amphotericin in 10 of 14 patients who had respiratory deterioration but in only one of eight patients who did not have respiratory failure. Our findings indicate that white blood cell treatment may cause changes in the lungs which amplify the usual toxicity of Amphotericin, thereby permitting severe pulmonary reactions. Amphotericin should be used very cautiously in patients receiving white blood cell treatment.

Significance to Biomedical Research and the Program of the Institute:

Our experiments with peripheral blood cell reconstitution in man extend the studies of Hershko *et al.*, in which isologous peripheral blood mononuclear cells were infused into a patient with paroxysmal nocturnal hemoglobinuria and aplastic anemia. In their experiment, no impact on the rate of peripheral blood leukocyte or lymphocyte recovery was observed following the infusion of isologous peripheral blood mononuclear cells. This difference is most probably related to the fact that immunosuppressive therapy was not employed in their study, and their patient had substantial numbers of circulating endogenous lymphocytes. In contrast, our patient had his lymphocytes, as well as granulocytes and platelets, suppressed by intensive myelosuppressive therapy. Both our experience and that of Hershko *et al.*, suggest that the clinical use of peripheral blood mononuclear cell transfusions with the intent of hematopoietic reconstitution should be approached with caution, especially in myelosuppressive settings. Further studies utilizing peripheral blood mononuclear cell populations enriched in CFUc (or other hematopoietic stem cells) in both preclinical and clinical settings would be of great interest in delineating the possible role of peripheral blood cells in hematopoietic reconstitution. We are presently engaged in such studies in both dogs and man in hopes of determining whether peripheral blood mononuclear cells collected at times of hematopoietic stem cell enrichment will reliably promote hematopoietic recovery following intensive myelosuppressive therapy in man. Our studies in patients with extensive small cell carcinoma of the lung utilizing cyclophosphamide, doxorubicin, VP16-213, and vincristine produced expansion of circulating CFUc numbers to levels greater than twice the pretreatment median in 95% of the patients studied when results are expressed as CFUc per 10^6 peripheral blood mononuclear cells, and in 85% of the patients studied when results are expressed as CFUc per ml of peripheral blood. The median level of expansion above the pretreatment

median was 7.9 fold when results are expressed per 10^6 peripheral blood mononuclear cells (range 0.9-157) and 6.7 fold when results are expressed per ml of peripheral blood (range 0.7-22.4).

Several Clinical Oncology Program protocols are designed to test the efficacy of intensive combined modality therapy either for the treatment of neoplasia resistant at relapse to conventional dose therapy, or in the adjuvant setting following the attainment of complete remission in neoplasia expected to relapse at a high frequency despite maintenance therapy. The safe administration of such intensive therapy is dependent upon the capability to utilize hematopoietic rescue so as to avert prolonged myelosuppression with its attendant risks of bleeding and infection. Accordingly, it was of importance to work out in animals the dose of autologous bone marrow cells, both in terms of total nucleated marrow cells as well as committed myeloid precursor cells (CFU_C), needed to ensure hematopoietic reconstitution in patients following intensive therapy. These doses of nucleated bone marrow and peripheral blood elements needed to ensure hematopoietic reconstitution have been used in the treatment of lymphoma, Ewing's sarcoma, soft tissue sarcomas, and small cell carcinoma of the lung (NCI-VA MOB Branch Study). The dog model has been used to establish the doses of peripheral blood mononuclear cells needed for hematopoietic reconstitution following intensive therapy. The existence of the dog model permitted us to translate a clinical observation made in patients with small cell carcinoma of the lung at the NCI-VA MOB (amplification of CFU_C in peripheral blood) into a new support modality which is being tested in the current clinical protocol for the adjuvant intensive combined modality therapy of small cell carcinoma of the lung. These studies have confirmed that amplification of pluripotent hematopoietic stem cell function occurs during the recovery of hematopoietic function following conventional dose therapy. They have shown that as few as two 4-hour collections of peripheral blood mononuclear cells are adequate in dogs for full hematopoietic reconstitution.

Another example of the critical role this canine model has played is in the elaboration of novel approaches to the treatment of resistant neoplasms. In particular, recent interest has developed in the use of immunoabsorbents to amplify or initiate an effective host response against tumor growth. Early reports on the effect of S. aureus as an immunoabsorbent, and tumor regression in animals following extracorporeal perfusion of the plasma of such hosts through columns containing S. aureus (Cowan strain) have led to the suggestion that this technique might be of use in the treatment of advanced cancer in patients. The existence of the preclinical canine model permitted us to test the therapeutic effectiveness of this maneuver in animals. We were able to document tumor regression in animals afflicted with mammary carcinoma, which confirmed the early data from other laboratories suggesting that this might be an effective treatment modality for some forms of cancer. These results have two major impacts on our program: If we continue to observe regression of tumors in dog carcinomas, we will initiate a phase I trial of this maneuver in man to assess its

toxicity, and later to evaluate its clinical efficacy in a phase II. We will also embark upon an investigation of mechanisms by which tumor regression is mediated with the maneuver.

Proposed Course:

We will continue to use preparations of autologous cryopreserved marrow for the support of patients receiving intensive therapy for resistant sarcomas (both soft tissues and bones), undifferentiated lymphomas, and extensive small cell carcinoma of the lung. In selected patients and in the animal model, we will also be able to study the interaction between stem cells from the marrow and peripheral blood on the recovery of myeloid and immune function following intensive therapy. A major extension of the studies in man on amplification of peripheral blood stem cell levels will be the testing of pharmacological manipulations to amplify these stem cells in the peripheral blood so that they may contribute to restoration of both immune and myeloid function following intensive therapy.

We will attempt to complete the current studies on the utility of various in vitro tests of compatibility on the success of platelet support of cancer patients.

Finally, we will attempt to analyze the use of hemoperfusion, using the continuous flow centrifuge and immunoadsorbents in the animal model and in clinical trials, to alter the course of growth of neoplasms refractory to other forms of treatment. This effort will involve the collaboration of a number of immunology laboratories which will be necessary to attempt to clarify the mechanism underlying the tumor regressions already seen with this treatment.

Appendix A

Contract Number: N01-CM-43702

Contractor: Hazelton Laboratories of America, Inc.

Principal Investigator: Charles Bowles

Budget: \$276,000 per annum

Man Years: 10 (4 = professional, 6 = other)

Objective:

Pre-clinical investigation of leukocyte transfusion, and autologous marrow or peripheral blood stem cell "rescue."

Findings:

The pre-clinical canine model was used to test the reconstitutive capability of peripheral blood mononuclear cells collected from animals during the period of hematopoietic recovery associated with amplification of peripheral blood levels of stem cells. These studies revealed a 10-fold increase of stem cells in the peripheral blood as outlined in the text. The model was also used to test the effect of extracorporeal immunoabsorption on canine mammary adenocarcinoma. Regressions of tumor were observed in roughly 50% of animals so treated.

Plan:

The pre-clinical canine model will be used to study the effect of pharmacological manipulation on the ability of peripheral blood cells to foster immune as well as myeloid recovery following intensive therapy. The interaction of peripheral blood and marrow cell infusions will be tested following intensive therapy on the recovery of hematopoietic as well as immune recovery. A major effort will also be made to improve the immunoabsorption model already studied using Cowan's strain of S. aureus. Different solid phases for the immunoabsorbent as well as different immunoabsorbents will be tested to adapt this treatment for use in man. In addition, experiments will be conducted to test the origin of the tumor response as well as the interaction of this immunological treatment modality with that of chemotherapy.

Publications:

See main report.

Appendix B

Contract: N01-CM-63810

Contractor: Microbiological Associates

Principal Investigator: Robert Trapani, Ph.D.

Budget: \$145,000 per annum

Man Years: 3.29 (2.22 - professional)

Objective:

To perform crossmatch studies prior to transfusion of platelets and leukocytes, and to store sera of Pediatric Oncology Branch patients.

Findings:

The POB maintains a serum repository for the serial storage of samples taken from patients undergoing chemotherapy and/or follow-up for treatment of a wide variety of neoplastic disorders. Over 18,000 entries are maintained at -80° C and are catalogued with respect to entry time, patient name and hospital number, disease, and volume of material remaining.

Plans:

The serum repository will be utilized for continuation of the retrospective and prospective trials previously described. In addition, we shall continue to collect samples from patients in anticipation of future clinical investigations. The conclusion of our prospectively randomized trial of HLA-matched vs. mismatched platelet transfusions is critically important to permit adjustment of funding priorities not only intramurally but extramurally as well. The serum repository as well as the in vitro tests of compatibility (lymphocytotoxicity, granulocytotoxicity, platelet migration inhibition) represent integral parts of this protocol.

Publications:

See main report.

Appendix C

Contract Number: N01-CM-02092

Contractor: UCLA

Principal Investigator: Paul I. Terasaki, Ph.D.

Budget: \$398,000 per annum

Man Years: 12.9 (8.1 = professional)

Objective:

This is a service contract which provides HLA-typing for patients, families, and blood donors (Clinical Center and Baltimore Cancer Research Center). The contract also provides the computer processing required for the national platelet donor pool.

Findings:

See narrative (06820) concerning numbers of donors HLA-typed and the status of the national donor pool.

Publications:

See main report.

Publications:

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2. Abrams, R., Glaubiger, D., Appelbaum, F., and Deisseroth, A.B.: Result of attempted hematopoietic reconstitution using isologous, peripheral blood mononuclear cells. *Blood*, in press.
3. Abrams, R., Glaubiger, D., Simon, R., Lichter, A., and Deisseroth, A.: Four week reduction of myelosuppression by autologous stem cell infusions in man. *The Lancet*, in press, 1980.
4. Abrams, R., Levine, A.S., and Deisseroth, A.: The Use of Intensive Anti-Neoplastic Therapy in Association with Hematopoietic Reconstitution: Rationale and Results. In Staquet, M. and Klastersky, J. (Eds.): Supportive Care in Cancer Patients. New York, Raven Press, in press.
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October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Infectious Complications of Malignancy: Prevention, Diagnosis and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:

6

PROFESSIONAL:

4

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2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection is the major cause of morbidity and mortality in patients with malignant disease. We are developing and evaluating the utility of various techniques and procedures to reduce the incidence of severe infection in cancer patients. The role of oral absorbable antibiotic prophylaxis is being evaluated in patients receiving standard doses of chemotherapy, and the role of protected environments (laminar air-flow room isolators) is being studied in patients receiving intensive chemotherapy. We are also evaluating new methods for detecting occult infections in neutropenic patients, e.g., gallium scanning in conjunction with white cell transfusion and the ELISA inhibition assay for fungal antigen. Empiric and specific antibiotic treatment of granulocytopenic patients with fever of unknown origin or proven infections is being prospectively studied, and the role of newer antimicrobial agents is also being investigated. The role of chemically-defined immunoregulatory agents is being evaluated in in vitro and in vivo models to develop methods of improving host defenses following immunosuppressive chemotherapy and reduce the incidence and severity of infectious complications.

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Objectives:

1. To assess the current etiologies of febrile episodes in patients with malignancy, and the relationship of these episodes to cancer treatment, prior infection, degree of host compromise, underlying disease, and granulocytopenia.
2. To determine the most appropriate means for the evaluation of fever in the compromised host by assessing invasive and noninvasive diagnostic techniques.
3. To improve methods by which occult infections may be detected and treated.
4. To assess the possibility of reducing the incidence of infectious complications in granulocytopenic cancer patients by the use of prophylactic antibiotics.
5. To assess the efficacy and toxicity of empiric and specific antibiotic regimens for patients who become febrile and/or infected during periods of granulocytopenia. The role of antifungal therapy in high-risk patients will also be determined.
6. To improve the utility of the "total protected environment" in reducing the incidence of severe infection in patients with various lymphomas and solid tumors who are undergoing very intensive chemotherapy (with or without autologous marrow rescue), and to learn whether the intensive treatment possible in this circumstance results in improved tumor response and prolongation of survival.
7. To evaluate methods for bolstering host defenses and stimulating granulocyte recovery following chemotherapy-induced immunosuppression as an adjunct to the treatment and prevention of infections.

Methods Employed:

Patients considered to be at high-risk for developing chemotherapy-induced granulocytopenia are randomly assigned to receive oral absorbable antibiotic prophylaxis or a placebo prior to the onset of granulocytopenia and fever. Patients are followed clinically to determine if subsequent fever (and/or infection) does occur, as well as to detect antibiotic toxicity and/or the development of microbial drug resistance. Patients who do become febrile while granulocytopenic are carefully assessed for the possibility of an infectious etiology and begun empirically on broad-spectrum antibiotics. Random assignment to subsequent antimicrobial therapy is stratified according to a number of variables in the pattern of fever and/or infection. All patients are closely monitored with pharmacokinetic and metabolic studies which relate to antibiotic levels and

(potential toxicity, and surveillance for changes in antibiotic sensitivity is carried out. Selected patients are the subject of newer diagnostic studies including gallium scintiscanning with or without leukocyte transfusions, ELISA-inhibition assay for Candida antigens, and assays for Enterobacteriaceae anti-core glycolipid. Assessment of the total protected environment [laminar-air-flow room (LAFR), oral nonabsorbable plus absorbable antibiotics, cutaneous and orificial antisepsis] includes serial patient and environmental surveillance as well as longitudinal assessment of physical compliance and psychological tolerance. Laboratory investigations of immunomodulating agents include lymphocyte blastogenesis and function studies, reverse plaque inhibition assays, in vitro bone marrow culture, and challenge with infectious agents.

Major Findings:

A. Evaluation and Management of the Febrile, Neutropenic Cancer Patient

Since November, 1975, we have prospectively evaluated all patients of the Pediatric Oncology Branch of the National Cancer Institute who developed a fever and granulocytopenia. In this study fever is defined as either three oral temperature elevations above 38°C during a 24-hour period, or a single oral temperature elevation of 38.5°C or higher. Granulocytopenia was defined as an absolute granulocyte count of less than 500 polys and bands per mm³. Patients meeting these criteria were admitted to the hospital and entered onto study. The initial evaluation of each patient included a careful history and physical examination; chest x-ray; urinalysis; routine blood chemistries; cultures of nose, throat, stool, and urine; and at least two pre-antibiotic blood specimens for routine and fungal culture. In addition, cultures of clinically probable or possible sites of infection (skin lesions, cellulitis, etc.) were aspirated and/or biopsied. Six-hundred-and-nineteen episodes (in 261 patients) have fulfilled the criteria of fever and neutropenia and have been entered into the study since November, 1975. A documented source of infection was definable within the first seven days in 308 (49.8%/o) of these episodes. The remaining 311 episodes (50.2%/o) had no detectable infectious etiology and were, therefore, classified as FUO's.

B. Observations Made on Patients with Documented Infections

1. Corynebacteria Sepsis - Septicemia with Corynebacterium diphtheriae and Corynebacterium equi have been recently described in cancer patients. Of additional concern is the observation that some recently described species of corynebacteria are resistant to virtually all antimicrobials except vancomycin (the drug of choice). Consequently, we studied the colonization of pediatric oncology patients with this organism to ascertain whether it was hospital-acquired and, if so, by which routes. Three groups were

studied (normal volunteers, pediatric oncology patients, and nonmalignant patients) at the time of their first hospital admission and were then followed during subsequent hospitalizations. Colonization of the inguinal and axillary area was demonstrable in 30-35% of newly admitted patients. Colonization was more common in males, and was associated with septicemia in 20% of the colonized cancer patients, usually as part of their terminal illness. Recognition of this organism, its prevalence, and antibiotic resistance is important in caring for oncology patients as well as treating potentially infected patients.

2. Treatment of Herpes Zoster infections - Over the past two decades, considerable experience has been accrued with longwave ultraviolet light in the A range (UVA: 320-400nm). When the oral photosensitizing furocoumarin derivative 8-methoxypsoralin is combined with UVA, the resulting "PUVA" regimen has been highly successful in the treatment of psoriasis and has also been used for vitiligo and mycosis fungoides. With longwave UVA, at least 60% of the light energy reaches the dermal layer. The mechanism of action of PUVA is thought to be related to the production of photoadducts with thymidine in DNA, leading to an inhibition of DNA synthesis in replicating cells. Interference with viral DNA has also been demonstrated with SV40. Recent experimental data using PUVA for Herpes simplex in a guinea pig model has been shown to be beneficial. The potential effect of PUVA for local and/or generalized H. zoster is unknown, although a single patient treated at the Massachusetts General Hospital had an encouraging response with PUVA therapy. While it is likely that the therapy will reach cutaneous sites, the ability of PUVA to reach deeper nerve tissue is speculative. The questions addressed in this study include the following: Are there advantages to PUVA therapy for cancer patients with H. zoster with regard to dermatomal spread, local pain, postherpetic neuralgia and extradermatomal dissemination? What are the side-effects of PUVA therapy in chemotherapy treated cancer patients? Are the metabolism and pharmacokinetics of 8-methoxypsoralin altered in patients who have had prior chemotherapy? For patients who present with generalized herpes infections, we plan to evaluate the utility of promising new antiviral agents (e.g., acyclovir). These studies will be done in conjunction with the NIAID and the Medicine Branch of the NCI.

C. Observations Made on Patients with Fever of Unknown Origin

1. Low and high risk FUOs - As noted above, infection cannot be proven in half of the patients who present with fever and granulocytopenia, defining these patients as FUO's. We have prospectively evaluated the antibiotic management of these patients and have observed that they can be divided into low and

high risk categories: The majority (61⁰/o) are low risk, as defined by the resolution of neutropenia (PMN>500/mm³) within seven days following the initiation of empiric antibiotic therapy. None of these patients developed recurrence of fever or other infectious complications when the antibiotics were discontinued at the resolution of neutropenia; hence, a short course of empiric antibiotic therapy appears safe for this group of patients. In contrast, FUO patients with prolonged neutropenia (PMN<500/mm³ for >7 days) should be considered in a "high-risk" category. In the NCI series, 39⁰/o of the FUOs were in this category, and these patients can be further subdivided into those who become afebrile (but who remain neutropenic) while receiving empiric antibiotic therapy, and patients who continue to remain febrile while receiving antibiotics. To test the advantages and disadvantages of continued antibiotic therapy in these patients, we randomly assigned high-risk FUO patients to receive or not receive continued antibiotic therapy. None of the FUO patients who had become afebrile but who remained neutropenic and who were randomized on day 7 to continue antibiotics (until the neutropenia resolved) developed subsequent infectious sequelae. In contrast, 41⁰/o of the afebrile-neutropenic FUO patients randomized to discontinue empiric antibiotics developed fever and/or infection within a median of two days after stopping antibiotics. These observations suggest that it is beneficial to continue empiric antibiotics in FUO patients who have become afebrile but who have prolonged neutropenia. Whether these antibiotics should be continued for the duration of the neutropenia, or for a limited (e.g., 14 day) treatment course, is currently being studied by randomizing patients remaining afebrile and granulocytopenic after 14 days of antibiotic therapy either to discontinue antibiotic therapy at that time or to continue until the granulocytopenia has resolved. Since the initiation of this study, 9 patients have been randomized on day 14: 5 patients to discontinue antibiotics and 4 patients to continue antibiotic therapy until the neutropenia has resolved (PMNs>500/mm³). Two granulocytopenic patients in whom antibiotics were discontinued again became febrile (one patient 2 days after the antibiotics were discontinued, the second 7 days later). These preliminary data suggest that antibiotics serve a prophylactic role in persistently granulocytopenic but afebrile patients.

Of the 92 granulocytopenic FUO patients who had become afebrile during the first 7 days of empiric antibiotics, 3 developed a new fever prior to the day 14 (day 10, 12 and 13) randomization. As prescribed by the protocol, all these patients were begun on Amphotericin B. All patients recovered, although two manifested retrospective evidence of fungal infection temporally associated with the second fever. These data suggest that empiric antifungal therapy is an important consideration for such patients.

2. The FUO with persistent fever and neutropenia:

The second high-risk FUO group are the patients who continue to remain both febrile and neutropenic after seven days of empiric antibiotics. For these patients, the possibility that the empiric antibiotic regimen is "controlling" an occult infection must be balanced against the possibility that the antibiotics are inadequate, or that a fungal infection is a primary or secondary complication. Hence, in addition to empiric antibiotic therapy, we are evaluating the additional utility of empiric antifungal therapy in these patients. This group was randomized on day 7 either to discontinue antibiotics, to continue antibiotics until both fever and granulocytopenia resolved, or to continue antibiotics with the addition of Amphotericin B until fever and granulocytopenia resolved. Preliminary observations reveal that 9 of the 16 FUO patients randomized to discontinue KGC developed an infectious complication after stopping antibiotic therapy, and that 6 of the 16 FUO patients randomized to continue KGC alone subsequently developed an infection (1 bacterial, 5 with disseminated fungal infection). In contrast, only 2 of 18 patients randomized to continue KGC and empiric Amphotericin developed demonstrable infectious sequelae, even though the median duration of neutropenia and continuous antimicrobial therapy was 24 days.

During this same three-year study period, patients in whom an infectious etiology was initially demonstrated but who remained febrile and neutropenic after 7 days of appropriate antibiotic therapy, and who were known to be colonized with candida throughout their gastrointestinal tract, were also begun on empiric Amphotericin. Twenty-two patients were treated in this category, the median duration of neutropenia and antimicrobial therapy being 26 days; each of these patients recovered without evidence of fungal infection. In contrast, 20 patients (out of 90 at risk) died with evidence of invasive fungal infection during the three-year study period. None of these patients had received early antifungal therapy: in 6 patients evidence for colonization was not obtained for 2 weeks after initiation of broad spectrum antibiotics; in 9 patients Amphotericin was not administered because of the state of the patient's underlying malignancy; in 5 patients Amphotericin was initiated only after evidence of invasive or disseminated fungal infection was present. Collectively, these data suggest that early empiric antifungal therapy may be beneficial for febrile patients with prolonged neutropenia who are receiving protracted antibiotic therapy, as evidenced by the diminished incidence of invasive fungal disease at postmortem examination during this study period.

We are also attempting to determine if certain risk factors or initial signs and symptoms predict whether a febrile-granulocytopenic patient has a documented infection or an

FUO. Furthermore, we have attempted to learn whether these factors would predict if the FUO patient would be low or high risk. In this regard we compared the historical features and presenting characteristics of patients whose outcome (FUO vs. infection) was determined. These data failed to reveal reliable predictive factors, underscoring the necessity for early empiric antibiotic therapy when the neutropenic patient becomes febrile. Clearly the development of non-culture dependent indicators of infection is necessary to provide a more sound basis for antimicrobial therapy in these patients.

D. Studies on the Prevention of Infection

1. Prophylactic treatment of granulocytopenic patients prior to the onset of fever - The objective of this trial is to reduce the incidence of fever and infection in patients who are likely to develop treatment-related granulocytopenia. In view of the value of systemic antibiotics in preventing infections in granulocytopenic patients who have been febrile, the possibility that similar agents might prevent fever (and infection) per primum is worthy of study. Accordingly, the prophylactic regimen employed should fulfill the following criteria: 1. Broad-spectrum coverage of both gram-positive and gram-negative organisms must obtain; 2. antibiotics must be used which are not routinely employed for the therapy of systemic infections (e.g., penicillin, semisynthetic penicillins, cephalosporin, and/or aminoglycosides); 3. a regimen should be employed which can be administered orally and with good patient acceptance. Sensitivity testing performed by the Clinical Center Microbiology Laboratory suggested that the combination of erythromycin and Bactrim (trimethoprim-sulfamethoxazole) would fulfill these criteria. Our study design is a placebo-controlled double-blind randomized trial for which patients are eligible who are receiving chemotherapy which predictably produces granulocytopenia ($PMN's < 500/mm^3$). This study was initiated in March, 1979. To date, 108 patients have been entered into the study and 307 episodes have been evaluated. The compliance of patients in taking the oral medications (or placebo) has been carefully monitored and patients are categorized as follows: 1. Excellent compliance - patient took all prescribed pills throughout the period of granulocytopenia; 2. good compliance - patient took medications regularly but missed occasional doses; 3. poor compliance - patients took pills irregularly or not at all. According to these criteria, excellent compliance has been observed in 68⁰/o of the episodes, good compliance in 16⁰/o and poor compliance in 16⁰/o.

At the conclusion of this trial, we should be able to answer the following questions: 1. Will patients receiving prophylactic antibiotics have a significant reduction in the incidence of fever and/or infection during periods of granulocytopenia? 2. Will the

administration of oral antibiotics make the diagnosis of infection more difficult should the patient still become febrile? 3. Will the patient, family, and staff comply with the prophylactic schedule? 4. Will the antibiotics affect the colonization pattern of the patient's flora, including the development of resistant organisms? 5. Will the patient's flora become altered with recurrent administration of the oral prophylactic regimen? 6. Will the patient's initial flora serve to predict response (or lack of response) to this prophylactic regimen? 7. Will organ toxicity (hepatic, renal, etc.) be encountered with this regimen? and 8, if effective in reducing infection, will these measures permit more optimal use of cancer chemotherapy? This same study is also being conducted (under our direction) in the Medical Oncology Division of the University of Florida. This collaboration complements our studies by providing an older study population for comparison.

2. Protected environment study - Patients whose tumor(s) have become resistant to standard doses of chemotherapy might respond to very intensive doses. Moreover, the use of intensive chemotherapy prior to the development of drug resistance in patients who present with widely disseminated tumor might result in an improved remission rate and/or duration of response. The major limitation to intensive chemotherapy, however, is severe bone marrow depression and the heightened risk of infection. The use of a Protected Environment (PE), consisting of laminar-air-flow room isolation in conjunction with nonabsorbable oral antibiotics, cutaneous antisepsis, and a diet with a low microbial burden, has previously been shown to reduce the incidence of infection related to prolonged granulocytopenia. The present study addresses the following questions: Can patients, including young children, tolerate prolonged periods of isolation and comply with the PE regimen? Second, can the PE regimen successfully reduce the incidence of infection in young patients with solid tumors, as it has in adults with leukemia, and permit the administration of more intensive chemotherapy? Finally, will such intensive drug regimens improve the remission rate and/or duration of response within the tumor spectrum currently seen in this Branch? Since September, 1975, 90 patients ranging in age from 2 to 33 years old have received intensive chemotherapy for a variety of pediatric solid tumors. Patients received varying 3-4 drug combinations which included vincristine, methotrexate, adriamycin, actinomycin, ara-C, 6-TG, mCCNU, DTIC, and/or L-PAM. In addition, each regimen included high-dose cyclophosphamide in doses ranging from 135-200 mg/kg over 3-4 days. These regimens resulted in periods of absolute granulocytopenia ($PMN's < 500/mm^3$) ranging from 15-66 days. Sixty of these patients received the intensive drug schedule in a PE, the remaining 30 patients being treated in a standard hospital room. Patients whose bone marrow had been cryopreserved were randomized to receive therapy in either the PE or normal

hospital room. Patients not receiving autologous bone marrow infusions were non-randomly assigned to the PE. Seventy-two of the 90 patients receiving intensive chemotherapy (80⁰/o) had failed prior treatment and were considered resistant to standard doses of chemotherapy. Fifty of these patients received treatment in a PE. The disease distribution among these patients included: non-Hodgkin's lymphoma (22), rhabdomyosarcoma (5), Ewing's sarcoma (5), osteosarcoma (11), neuroblastoma (3), and leukemia (3).

Overall, a documented infection has occurred in 9/60 (15⁰/o) of PE patients and in 11/30 (37⁰/o) of the control patients. Death due to infection occurred in 2/60 (3⁰/o) of the PE patients and in 3/30 (10⁰/o) of the control group. In addition, 6/60 (10⁰/o) PE patients died as a result of extramedullary toxicity following intensive therapy compared with 6/27 (22⁰/o) of the control patients. Regardless of where the treatment was administered, approximately 50⁰/o of the patients had a complete or partial response, but the median duration of remission for these patients was brief (approximately 2 months) and only 10⁰/o achieved a long-term survival. Nonetheless, the fact that some of these tumors were responsive to very intensive chemotherapy suggests that these regimens may be more useful in patients prior to the development of overt drug resistance.

Since certain pediatric solid tumors are characterized by early drug responsiveness but early relapse (especially when the patient presents with metastatic tumor), it is possible that early intensive therapy (i.e., prior to drug resistance) will increase the duration of response. This hypothesis is being tested in patients presenting with metastatic rhabdomyosarcoma and patients with central axis or metastatic Ewing's sarcoma. Eighteen patients (6 rhabdomyosarcoma and 12 Ewing's sarcoma) have been treated in this fashion to date. Ten of these patients received intensive chemotherapy in a PE, 8 in a standard hospital room. The preliminary observations in these patients are encouraging. Clearly the ultimate utility of these regimens depends on whether they yield a significant improvement in disease-free survival among such high-risk patients.

We have also evaluated the degree to which pediatric patients comply with the PE regimen in adhering to the nonabsorbable antibiotic schedule. Of 45 patients evaluated, 18 (40⁰/o) showed excellent compliance, 9 (20⁰/o) good compliance and 18 (40⁰/o) poor compliance. Younger patients (<8 yrs) were generally more compliant than adolescents. Surveillance microbiology shows that the current antibiotic regimen completely suppresses the resident flora in only 29⁰/o of the patients while 24⁰/o have persistent bacterial flora and 47⁰/o of the patients remain colonized. To improve the decontamination regimen and to further reduce the

incidence of febrile episodes in PE patients, we are presently evaluating the benefit of adding bactrim-erythromycin to the PE regimen by randomizing patients to receive or not receive these antibiotics in addition to the routine and nonabsorbable antibiotic regimen. To date 8 patients have entered this substudy, four in each arm. We will also evaluate the value of the imidazole antifungal agents in reducing candida colonization.

We have also studied the possibility of modifying and simplifying the low-microbial diet necessary for PE patients. The present cooked-food diet is bland and may become intolerable for patients undergoing prolonged periods of isolation. We have evaluated more than 200 commercially available canned and frozen food products as well as dairy products, meats, and fresh fruits for their microbiologic content and ease of preparation. More than 680/o of these items are, by our criteria, microbiologically safe (i.e. <500 Bacillus colonies per gram or cc) and provide a dietary alternative for isolated patients.

In conjunction with NIMH, extensive psychological testing, observation, and monitoring has been conducted on PE isolated patients and their families. The following conclusions can be drawn: Isolation of young children (<5 yrs), even when extended over months, does not impede normal developmental milestones. Moreover, social interaction by children and adolescents continues at a high level in isolated patients, even during physical illnesses (i.e., fever and/or infection). Nonetheless, depression is a frequent occurrence in isolated adolescents, usually commencing with physical symptoms, but generally continuing beyond the resolution of physical symptoms. Pre- and post-isolation interviews with children and adolescents show them to be generally well informed about their medical illness and its consequences, but nearly always with an unexpectedly high level of optimism - an attitude which is important for medical staff to be aware of and to support.

E. Experimental Models for Enhancing Host Defenses Following Chemotherapy-Induced Immunosuppression

Empiric and prophylactic antimicrobial schedules have contributed significantly to reduce infectious morbidity and mortality but their potential is likely to be limited, and their prolonged use is not without toxicity (e.g., organ damage and superinfections). Clearly, improved treatment schedules will require development of less toxic antimicrobial agents in conjunction with improved methods of bolstering host defenses. In recent years it has become recognized that several diverse substances have immunomodulating effects. These agents ("immune-adjuvants") may augment normal immune responses, or may restore or override impaired and depressed host-defense responses.

Little is known regarding the mechanisms involved in this apparently non-specific enhancement induced by immune adjuvants. Moreover, these agents, (e.g., BCG, C. Parvum) may produce severe local and systemic side effects, thus restricting their clinical application. Consequently, it is of interest that several chemically-defined agents (e.g., lithium, glucan, bestatin, etiocholanolone, muramyl dipeptide, 2-cyano-substituted aziridines) have similar immunostimulatory properties and also appear to interact with the hematopoietic system. These agents offer a unique opportunity to explore hematopoiesis, and by pharmacologic manipulation, offer the further possibility of stimulating immunologic and hematopoietic recovery during chemotherapy-induced immunosuppression, affording new modalities for host protection. The overall aim of our studies is to define the relative efficacy of these (and other) immunoregulatory chemicals in stimulating or enhancing granulopoiesis and immunoreconstitution. These studies are being conducted in animal models as well as in an in vitro marrow culture system in order to: a) Define the effect of these agents on leukocyte kinetics and cell-mediated immunity; b) maximize the dose and schedule of administration to enhance granulopoiesis and other immune parameters; c) assess the efficacy of these agent(s) in immunosuppressed rodents; d) evaluate the role of these agents in protecting immunosuppressed rodents from infectious challenge; e) determine whether combinations of immune-regulatory agents have an additive augmentation of host recovery and protection from infection; f) compare the effects of these agents on the proliferation of tumor cells vs. hematopoietic stem cells to assess differential sensitivity and specificity; g) determine the mode of action of particularly promising agents; h) and to evaluate the combined utility of bolstering host defenses with appropriate prophylactic antibiotics in the protection from infection and in permitting the design of chemotherapy schedules which have improved therapeutic efficacy. Naturally the ultimate goal of these studies will be to apply promising results to clinical trials.

Significance to Biomedical Research and the Program of the Institute:

Infection remains the leading cause of morbidity and mortality in the cancer patient. The majority of infections occur as a consequence of disease and/or treatment-induced alteration of host defenses (especially granulocytopenia), and they are the major impediment to the delivery of cancer chemotherapy. Consequently, effective supportive management of the patient is essential if the potential benefits of chemotherapy are to be achieved. This includes an understanding of the natural history of infectious complications in the compromised host, especially their early recognition and diagnosis. Our studies to date have helped to define the appropriate evaluation of the febrile, neutropenic cancer patient, as well as the specific management of particular infections and fevers of undetermined origin. These changes in management have resulted in a significant reduction in the morbidity and mortality related to infection.

Moreover, our studies of infection prevention (both the protected environment and empirically administered antibiotics) suggest that the frequency and morbidity of infectious complications can be significantly reduced, thus permitting the optimal delivery of cancer chemotherapy.

Proposed Course:

We shall continue our studies of the natural history of infectious complications in cancer patients as outlined in the progress report, since this evaluation will help further to define high-risk patients and assist in their diagnosis and management. Rapid diagnostic assays which do not depend on culture of the organisms will be studied in order to provide the most rational basis for immediate antibiotic and anti-fungal management. Our studies on the optimal empiric use of antibiotics in febrile, neutropenic patients will be continued, as will our clinical trials related to the specific management of septicemia, local bacterial infections, and viral, protozoan, and fungal complications. Our studies on antibiotic prophylaxis will be continued, and the use of chemical or immunological adjuvants which might shorten the period of granulocytopenia will be assessed. Similarly, our studies of the protected environment will be continued, with emphasis on the early intensive treatment of high-risk tumors prior to the emergence of chemotherapy resistance.

The ultimate challenge is the development of effective cancer treatment methods which are tumor-specific and which do not produce the significant compromise of host defenses which result in infectious complications. However, until this goal is realized, we will continue to investigate more effective and less toxic methods for treating and preventing infection in immunosuppressed patients. Our major emphasis will be directed at prevention. We will seek more effective methods for suppressing and/or eliminating the host's endogenous microbial flora with absorbable and nonabsorbable antimicrobial agents. Our major research target will be to develop methods for immunostimulation of the host's defenses during chemotherapy-induced immunosuppression. Methods to activate cellular and humoral immunity, the macrophage-monocyte system, and mechanisms which expand and/or protect the neutrophil mass following chemotherapy will be sought through the use of chemically-defined immunoregulatory agents. While combining these host bolstering techniques with prophylactic antibiotics, we will also explore chemotherapeutic schedules which may have a more selective effect on tumor cells.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06840-05 P0																																							
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<table style="width:100%; border: none;"> <tr> <td style="width:10%;">PI:</td> <td style="width:70%;">D.G. Poplack, Senior Investigator</td> <td style="width:20%; text-align: right;">PO C</td> </tr> <tr> <td>Other:</td> <td>G. Reaman, Asst. Prof.</td> <td style="text-align: right;">G.W. Univ.</td> </tr> <tr> <td></td> <td>J. Blatt, Clinical Associate</td> <td style="text-align: right;">PO C</td> </tr> <tr> <td></td> <td>M. Trigg, Clinical Associate</td> <td style="text-align: right;">PO C</td> </tr> <tr> <td></td> <td>R.M. Blaese, Head, Cellular Immunology Section</td> <td style="text-align: right;">MET C</td> </tr> <tr> <td></td> <td>S. Broder, Senior Investigator</td> <td style="text-align: right;">MET C</td> </tr> <tr> <td></td> <td>W. Pichler, Visiting Scientist</td> <td style="text-align: right;">MET C</td> </tr> <tr> <td></td> <td>J. Smyth, Department of Medicine</td> <td style="text-align: right;">Royal Marsden Hospital</td> </tr> <tr> <td></td> <td>J. Donlon, Clinical Associate, Hematology Service</td> <td style="text-align: right;">CP CC</td> </tr> <tr> <td></td> <td>F. Bollum, Chairman, Dept. of Biochem.</td> <td style="text-align: right;">Bethesda Naval Hospital</td> </tr> <tr> <td></td> <td>S.K. Ladisch, Assist. Prof.</td> <td style="text-align: right;">UCLA</td> </tr> <tr> <td></td> <td>T. Callahan, Investigator</td> <td style="text-align: right;">LP C</td> </tr> <tr> <td></td> <td>R. Billing, Assoc. Prof.</td> <td style="text-align: right;">UCLA</td> </tr> </table>			PI:	D.G. Poplack, Senior Investigator	PO C	Other:	G. Reaman, Asst. Prof.	G.W. Univ.		J. Blatt, Clinical Associate	PO C		M. Trigg, Clinical Associate	PO C		R.M. Blaese, Head, Cellular Immunology Section	MET C		S. Broder, Senior Investigator	MET C		W. Pichler, Visiting Scientist	MET C		J. Smyth, Department of Medicine	Royal Marsden Hospital		J. Donlon, Clinical Associate, Hematology Service	CP CC		F. Bollum, Chairman, Dept. of Biochem.	Bethesda Naval Hospital		S.K. Ladisch, Assist. Prof.	UCLA		T. Callahan, Investigator	LP C		R. Billing, Assoc. Prof.	UCLA
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COOPERATING UNITS (if any) Metabolism Branch (NCI); Department of Medicine, Royal Marsden Hospital; Clinical Pathology Department (CC); Bethesda Naval Hospital; UCLA; Lab. of Path., NCI; Children's Hospital G.W. Univ., Washington, D.C.																																									
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SUMMARY OF WORK (200 words or less - underline keywords) In this project, mechanisms of normal cellular immunity and tumor immunology are studied. Immunological surveillance of cancer patients and the effects of chemotherapy and immunotherapy on the immune response are explored. We are characterizing acute lymphocytic leukemia according to the immunological subtypes of lymphoblasts and the capacity of lymphoblasts to express immunoregulatory helper and/or suppressor functions in vitro. Possible correlation between lymphoblast cell surface markers with various biochemical characteristics is being pursued. Particular emphasis is placed on immunologic study of the monocyte-macrophage system (including development of new clinical assays of human monocyte function) in malignancies and immunodeficiency diseases, in collagen diseases, and in the histiocytoses. We are also investigating serum suppressors of cellular immunity in cancer patients. The therapeutic and diagnostic potential of anti-leukemia antisera and leukemia-specific hybridoma antibodies is being evaluated in vitro and in vivo.																																									

Objectives:

1. To develop methods of distinguishing between immunological subtypes of leukemic lymphoblasts and further to characterize such subtypes.
2. To define possible correlations between immunological cell surface markers and various biochemical characteristics of leukemic lymphoblasts.
3. To study the biochemical status of the purine enzyme pathways in malignant lymphoid conditions in an attempt to assess their role in lymphoid differentiation.
4. To assess whether acute leukemic lymphoblasts are capable of manifesting immunologic "helper" and/or "suppressor" function, using in vitro immunologic assays.
5. To evaluate heterologous anti-leukemic antisera and hybridoma-produced antibodies as potential diagnostic and therapeutic tools for the treatment of acute lymphoblastic leukemia.
6. To examine the role and function of T cell subsets in normal and disease states.
7. To explore the role of the monocyte-macrophage in human disease states and to assess the functional heterogeneity of this cell system during normal maturation.
8. To study the mechanism(s) of lipid-induced plasma suppression of immune function.
9. To assess the effect of chemotherapy and immunotherapy on the host immune response.

Methods and Major Findings:A. Immunological and Biochemical Correlations Involving Leukemic Lymphoblasts

The categorization of lymphoid malignancy has largely been based on histological classification. In acute lymphoblastic leukemia in particular, recent immunological developments have allowed the classification of this disease on the basis of immunological subtypes. A significant proportion of our recent research efforts has been aimed at elucidating biochemical markers of potential diagnostic and therapeutic value. Specifically, our studies have involved 5 enzymes: adenosine deaminase, 5'-nucleotidase, purine nucleoside phosphorylase, terminal deoxynucleotidyl transferase and acid phosphatase. We have investigated the status of these enzymes in acute lymphoblastic

leukemia, defining where possible the correlation with currently utilized immunological classifications. Our results suggest that a "biochemical profile" of acute lymphoblastic leukemia cells may provide a mechanism for further defining subsets of ALL and may offer an avenue for new diagnostic and therapeutic approaches. These studies are being extended to other lymphoid malignancies.

1. Adenosine Deaminase (ADA) - The enzyme adenosine deaminase has been shown to have specific importance for the metabolism of lymphocytes. ADA activity of acute leukemia lymphoblasts is greatly increased in comparison with normal lymphocytes. We studied leukemic lymphoblasts to determine whether a correlation exists between ADA activity and immunological subsets of ALL. We studied ADA activity in lymphoblasts of 23 ALL patients and lymphocytes from 10 controls. ADA activity was assayed by a spectrophotometric assay. For the leukemic population, the mean ADA activity was 58 adenosine deaminase units/mg protein (range: 7.5 - 161.5 U) compared with a mean of 14 U in control lymphocytes (range: 6.4 - 37.5 U). For this entire group of ALL patients, the mean ADA activity was 4 times greater than in control lymphocytes. The ADA activity in the T cell ALL patients ranged from 32.0 - 161.5 ADA units/mg protein. The ADA activity was 3 times higher in T lymphoblasts (102 U) than in null lymphoblasts (30 U). This difference is highly significant ($P < 0.02$). Thus, in this study we confirmed that ADA activity is elevated in acute leukemic lymphoblasts and that there is considerable variation in enzyme activity among different patients. Immunological and biochemical subclassification of these patients on the basis of their cell surface markers and ADA levels demonstrated a highly significant positive correlation between T cell ALL and high ADA activity. Recently, it was shown that the inhibition of lymphocyte ADA levels impairs lymphocyte function in vitro, and causes lymphocytic toxicity in vivo. The use of potent inhibitors of ADA, such as 2'-deoxycofomycin, may provide a new and specific approach to the therapy of T cell ALL, a disease which is usually resistant to currently available chemotherapy. A phase I trial of this agent is currently being undertaken in our Branch.
2. 5'-Nucleotidase - is an ectoenzyme that catalyzes the dephosphorylation of 5'-nucleotides to produce the corresponding nucleosides. The function of this enzyme in vivo is uncertain, but in cells such as lymphocytes that do not synthesize purines de novo, 5'-nucleotidase is postulated to provide transportable metabolites. We measured the activity of this enzyme in the leukemic lymphoblasts of 23 patients with untreated ALL and correlated the results with conventional cell surface markers. 5'-nucleotidase was measured by a modification of the method of Shenoy and Clifford. The mean 5'-nucleotidase activity of null (non T, non B) lymphoblasts was 0.029 nanomoles AMP degraded/10⁶

cells/min which was comparable to that observed in normal lymphocytes (0.039 nanomoles AMP degraded/ 10^6 cells/min). However, a 10-fold reduction in mean 5'-nucleotidase activity was observed in T cell lymphoblasts (0.004 nanomoles AMP degraded/ 10^6 cells/min). This difference was statistically significant ($P < 0.001$). To determine whether the reduced activity of 5'-nucleotidase was associated with cellular commitment along the normal T cell immunologic axis, the enzymatic activity of purified normal T cells was measured and compared with activity observed in unseparated peripheral blood lymphocytes. There was no difference in the mean activity of 5'-nucleotidase between T lymphocytes (0.043 nanomoles of adenosine 5'-monophosphate degraded per 10^6 cells/min) and unseparated lymphocytes (0.042 nanomoles degraded). Not only did T cell lymphoblasts exhibit markedly reduced 5'-nucleotidase activity, but the lowest enzymatic activity was observed in lymphoblasts forming the largest numbers of erythrocyte rosettes. Thus reduced enzymatic activity of leukemic lymphoblasts correlated with the degree of expression of T cell surface markers. The putative role of membrane bound 5'-nucleotidase is to convert purine nucleotides to nucleosides which are then actively transported across cell membranes. It is unclear whether the absence of this enzyme in leukemic cells merely signifies membrane phenotypic atyp or represents a particular biologic advantage or disadvantage for these cells. The finding of reduced 5'-nucleotidase in T cell leukemic lymphoblasts, together with our previous observation of an increase in adenosine deaminase in T lymphoblasts, suggests the existence of a specific purine metabolic abnormality in these cells.

3. Correlation of Purine Nucleoside Phosphorylase with cell surface markers of lymphoblasts - Purine nucleoside phosphorylase (PNP) acts sequentially with adenosine deaminase in reversibly converting inosine to hypoxanthine. Like adenosine deaminase, purine nucleoside phosphorylase has a demonstrated role in normal lymphoid development. Selective cellular immunodeficiency characterized by specific abnormalities in T cell immunity have been noted in patients with PNP deficiency. We evaluated the lymphoblasts of patients with ALL for the presence of nucleoside phosphorylase activity. PNP was measured in leukemic blast cells from patients with ALL and the results were correlated with conventional immunologic cell surface marker studies on these lymphoblasts. The PNP activity was assayed spectrophotometrically by the coupled xanthine oxidase method. Ficoll-separated lymphoblasts obtained either by leukopheresis or bone marrow aspiration prior to treatment were studied. The median PNP activity of cells from 14 patients with null cell ALL (non T, non B) was 83.5 units (units expressed as Δ O.D. 293 in 1 min/mg/ml protein) which was comparable to that observed in 20 normal controls (82.0 units). In contrast, in 8 patients with T cell ALL, a median PNP activity of

39.0 units was observed. This difference was statistically significant ($P < 0.01$). Thus, the lymphoblasts from patients with T cell ALL demonstrate decreased nucleoside phosphorylase activity. In addition to providing another criterion for classifying subsets of ALL, these differences again suggest an abnormality in purine metabolism specific for T cell ALL. The exact cause of the abnormality and whether it reflects a primary or secondary abnormality in these cells is as yet unclear.

4. Purine Pathway Enzymes in Lymphoid Malignancy - Our experience evaluating the purine pathway enzymes in acute lymphoblastic leukemia has led us to study these enzymes in other lymphoid malignancies in an effort to determine whether there are different enzyme patterns among the disorders. We have studied the activities of PNP and ADA, enzymes known to play a role in lymphocyte differentiation, in cells from patients with Sezary Syndrome (SS), Acute Lymphocytic Leukemia (ALL) and B cell lymphoma (BL), as well as in normal peripheral blood mononuclear cells (PBMC) and thymocytes. Assays for PNP and ADA were done spectrophotometrically, following the decay of adenosine at 265nm or the formation of uric acid at 293nm. Results were expressed in units which represent the ΔOD in one minute/mg protein. Cell surface markers were assessed by standard immunologic techniques.

Cell Type	PNP*	ADA*	No. of samples
BL	100(30-200)	26(4-130)	14
PBMC	83(10-230)	15(0-58)	21
Null ALL	79(20-140)	60(10-110)	15
T cell ALL	38(10-100)	130(40-420)	9
SS	38(10-111)	8(0-50)	17
Thymocytes	30(20-35)	100(90-120)	3

*mean (range) in units

As shown above, PNP activity in both T-ALL and SS, though comparable to that in thymocytes, was significantly lower than that in null ALL, BL or PBMC ($p < 0.0003$). ADA activity was significantly higher in T-ALL than in null ALL and PBMC ($p < 0.02$). Of those cells which expressed T cell markers, ADA was significantly higher in T-ALL and thymocytes than in the more mature Sezary cells ($p < 0.005$). Our results provide evidence for distinct enzyme patterns among lymphoid malignancies and suggest that these may reflect differences in the stages of lymphocyte, particularly T cell, differentiation.

B. Study of Heterologous Antisera in Acute Lymphoblastic Leukemia

In collaboration with Drs. Ronald Billings and Paul Terasaki, we are evaluating such heteroantisera: 1) To determine their usefulness as an aid in the subclassification of ALL, 2) to assess their potential role

in increasing diagnostic sensitivity in the assessment of bone marrow relapse, and 3) as a possible means of in vitro immunotherapy for this disease. Our initial experiments have involved study of the reactivity of lymphoblasts obtained from untreated ALL patients against heteroantisera raised in rabbits. We have assessed four antisera to date, the two most important being an anti T-cell heteroantiserum and an antiserum to non-T, non-B lymphoblasts. These antisera were developed in rabbits using an approach that had proven successful in the development of antiserum against mouse leukemia. The appropriate immunizing cells are precoated with antilymphocyte serum (ALS) prior to injection into rabbits. The passively administered ALS is believed to block the response to normal antigens on leukemia cells by masking normal antigens rather than by centrally suppressing antibody synthesis. Antisera produced by this method are then tested for specificity by complement dependent cytotoxicity and immunofluorescent techniques. In this manner, with only minimal absorption, sera have been developed which react mainly with T cell ALL lymphoblasts. Similarly, specific "non-B, non T" ALL heteroantisera have also been developed. In addition to studying these two antisera, we have tested the reactivity of our own ALL patients' blast cells against two other antisera, an anti-I_a antigen antiserum (raised to papain digests of cell membranes from cultured B cells) and an antiblast cell antiserum (developed by immunizing rabbits with a lymphoblastoid ALL cell line, CCRF-CEM, subsequently absorbed with CLL cells). A significant characteristic of the anti-T cell and anti "ALL" antisera is that they both have been demonstrated, using in vitro bone marrow stem cell assays, not to be inhibitory to normal marrow CFU-C.

The lymphoblasts from 20 POB ALL patients have been evaluated with these antisera utilizing a complement dependent cytotoxicity assay. Seven of these were E rosette positive ALL, the remainder were non B, non T cell ALL (according to classic immunologic cell surface marker studies). The anti T cell antisera reacted with lymphoblasts from 6/7 E rosette positive patients. Lymphoblasts from 11/13 patients who were non-B non-T by conventional markers were positive with the "anti ALL" antisera. Lymphoblasts from two patients were negative to both anti-T and anti-ALL antisera. All but 3 of the patients' lymphoblasts reacted with anti I_a antisera. The exceptions were 3 individuals who were E rosette and anti-T cell antisera positive. As anticipated, all of the patients' lymphoblasts reacted with the anti blast cell antisera. The results thus far demonstrate good correlation between the reactivity with antisera and conventional surface marker studies, and they are consistent with the originally assessed specificities for these antisera. Although it is not possible to say at this time whether the different patterns of heteroantisera reactivity will correlate with biological differences between lymphoblasts (i.e., enzyme differences), this and other potential correlations (i.e., with prognostic factors) are under careful study.

Our available anti-T and anti-ALL antisera are not toxic to marrow CFU-C formation; consequently we are planning to evaluate the feasibility of in vitro immunotherapy with these antisera. The design of our studies will be to treat autologous marrow, obtained from patients in remission, with the appropriate anti-T or anti-ALL heteroantisera (which are non-toxic to stem cells). At relapse, individuals will be treated with ablative therapy, following which they will be re-infused with their "in vitro treated" bone marrow. Our intention is to test this hypothesis in those high risk ALL patients whose lymphoblasts show reactivity with the appropriate antiserum. Of our 20 ALL patients whose lymphoblasts we have screened with antisera, 7 are alive in complete remission. We plan to include these patients in an initial pilot study. Remission bone marrows from this cohort of individuals will be treated and stored for possible use in the event of subsequent bone marrow relapse.

C. Role of the Macrophage in Leukemogenesis

In collaboration with Drs. Allen Oliff and E. Scolnick of the Laboratory of Viral Diagnosis, we are studying the role of the mononuclear phagocyte in the development of Friend-virus induced leukemia. We have been studying a particular viral strain which, 6-8 weeks following injection into newborn mice, results in the development of leukemia. In contrast, this same virus injected into adult mice produces no leukemia. In an effort to determine if the susceptibility of the newborn animal to viral infection (and subsequent leukemia) is based on an immunologic mechanism specifically involving a defect in macrophage function, we have performed a number of preliminary studies. We have documented that administration to adult mice of agents known to paralyze macrophage function (such as silica) renders older animals equally susceptible to the development of leukemia. This observation strongly suggests that defective macrophage function is necessary for leukemogenesis. Studies currently in progress involving cell transfer protection experiments (e.g., pretreatment of newborn mice with adult macrophages) may confirm the importance of intact macrophage function in this animal model. These studies have stimulated us to consider a more thorough evaluation of mononuclear phagocytic function in our leukemia patients.

D. Studies of Fc Receptor Expression in Normal Human Lymphocytes

Recent investigations have demonstrated a physical transition of Fc receptor expression in Fc-IgG receptor - positive T cells (T_H) following interaction with IgG immune complexes. The mechanism by which this transition occurs apparently involves membrane patching and capping with resultant endocytosis and/or shedding of the receptor-ligand complexes. We have studied the role of cytoskeletal organelles (microtubules and microfilaments) in this transition phenomenon. Drugs such as colchicine and cytochalasin B, which are known to interfere with assembly of microtubules and microfilaments,

inhibit the transition in normal peripheral blood human T lymphocytes. Both drugs have a dramatic effect on the alteration of Fc receptor expression following immune complex interaction. Both colchicine and cytochalasin B treated T cells demonstrate retarded Fc-IgG receptor loss after immune complex exposure and abrogation of subsequent Fc-IgM receptor (T_H) expression. The observation that these agents exclusively inhibit T cells undergoing membrane modulation suggests that these drugs might affect cell structures which are involved only with the process of transition. It is of additional interest that investigation of this phenomenon in acute leukemic lymphoblasts (both T and non T, non B phenotypes) has revealed that these cells, unlike normal T lymphocytes, show only a slow loss of Fc IgG receptors after short term in vitro culture, and do not manifest increased expression of Fc IgM receptors. The exact reason for this apparent inability to manifest the normal transition from Fc-IgG to Fc-IgM receptors in culture is unclear and is currently being investigated.

We have also investigated the in vitro radiosensitivity of T cell subsets (T_H and T_H cells). T_H cells appear to be considerably more radiosensitive than T_H cells. Since the T_H cells are believed to be primarily involved in cytotoxic effector functions, while T_H cells subserve suppressor functions, our observations on the radiosensitivity of these T cell subsets may have some bearing on the nature of the immune defect which occurs following irradiation in vivo. In current studies, we are investigating the role of the T_H and the T_H subsets in in vitro bone marrow cultures.

E. Study of Lipid-Associated Plasma Suppression of Immune Function

In an immunologic evaluation of children with the various histiocytic syndromes, we observed that individuals with Familial Erythrophagocytic Lymphohistiocytosis possess an abnormal immunosuppressive factor in their plasma which appears to be a lipid moiety. Our studies of the immunosuppressive properties of this plasma lipid, and previous observations of the immunosuppressive properties of the various plasma lipid moieties, led us to investigate the effect of fat emulsions, used in intravenous hyperalimentation regimens, upon the immune system. Thus, we investigated the effect of intralipid on lymphocyte function. We found that the blastogenic response of normal human peripheral blood leukocytes was markedly inhibited in a dose-related manner. 1000 mg⁰/o of intralipid caused up to 90⁰/o inhibition of responses to specific antigen (such as SKSD and candida) and allogeneic cells in in vitro lymphocyte transformation assays. In contrast, the responses to non-specific mitogens were virtually unaffected. Short term preincubation with intralipid caused inhibition which was reversible by cell washing; however, long term preincubation or higher intralipid concentrations resulted in increasingly irreversible inhibition. In an effort to extend these observations, we obtained plasma from patients receiving intralipid infusions as part of

a hyperalimentation protocol. Post infusion plasma caused up to 100% suppression of the response of normal peripheral blood lymphocytes to specific antigens and allogeneic cells, when preinfusion plasma was used as a control. The inhibitory effect of intralipid was shown not to be due to lymphocyte cytotoxicity, alterations in the kinetics of the blastogenic response, or changes in the optimal concentrations of stimulant. Thus, in these studies, we were able to demonstrate that concentrations of intralipids, achieved in vivo after intravenous infusion, depressed in vitro lymphocyte blastogenesis. Although the exact mechanism of the lipid induced suppression of in vitro lymphocyte blastogenesis is as yet unclear, it appears as though certain macrophage dependent reactions are more susceptible to this lipid-induced immunosuppression. The data obtained in our studies points to the possibility of an adverse effect of intralipid on immunologic function in patients receiving intralipid hyperalimentation. Further studies to evaluate the possible mechanisms of the intralipid induced immunosuppression are in progress.

Significance to Biomedical Research and the Program of the Institute:

Our studies on the characterization of leukemic cells using immunological and biochemical markers have revealed the presence of distinct biochemical differences among leukemic lymphoblasts of different immunologic subclasses. In addition, extension of these studies into other lymphoid malignancies has revealed that development of a biochemical profile may provide a useful mechanism for classifying lymphoid malignancies in terms of their state of lymphoid differentiation. These findings may be of both prognostic and therapeutic significance. In addition, evaluation of the immunological functions of these cells, e.g., immune suppression and Fc receptor status, is providing valuable insight into the degree of differentiation of the various leukemic lymphoblast populations. Our studies of the utility of specific anti-leukemic antisera and hybridoma antibodies may yield information helpful in the diagnosis and treatment of acute lymphoblastic leukemia.

Studies in recent years have shown that the macrophage-monocyte system plays a central role in the host defense against tumor and infection. The development of assays of human monocyte function which are capable of detecting specific clinical abnormalities is of singular importance in furthering our understanding of the monocyte-macrophage system in human disease.

Our studies on the role of the mononuclear phagocyte in leukemogenesis in the Friend leukemia virus system, although preliminary, may confirm a major role for this cell type in the leukemogenic process, underscoring the need for further definition of the role of the mononuclear phagocytic system in patients with leukemia.

Proposed Course:

We are planning to expand our efforts to characterize acute leukemic lymphoblasts both biochemically and immunologically. In particular we are extending our evaluation of the role of the purine pathway enzymes not only in acute lymphoblastic leukemia but also in other lymphoid malignancies. Preliminary data suggest that determination of a biochemical profile by assessment of these enzymes may yield important information as to the state of maturation and differentiation of both normal and malignant lymphoid conditions. We are also planning to pursue the prognostic and therapeutic relevance of our findings. Our interest in heteroantisera to acute leukemic lymphoblasts has been expanded. Specific antileukemic hybridoma antibodies are being developed which may be of use in the diagnosis and the treatment of acute lymphoblastic leukemia. Investigation into the role of the macrophage - monocyte in both normal and disease states will include further studies examining the functional heterogeneity of this system as well as the biochemical nature of these cells during the activation process. We are pursuing our studies on the role of the mononuclear phagocyte in leukemogenesis in the Friend virus leukemia system. These studies should yield important information regarding the role of the mononuclear phagocyte in the initiation of the malignant process. Immune surveillance of patients with malignancies will continue with special emphasis on the assessment of the mononuclear phagocytic system.

Publications:

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TITLE OF PROJECT (80 characters or less) DNA and RNA Tumor Viruses: Molecular Mechanisms of Infection and Carcinogenesis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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P. Howley, Senior Invest.		LP C
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S. Gupta, Visiting Fellow		PO C
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LAB/BRANCH Pediatric Oncology Branch		
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) We are studying the organization and expression of SV40 and <u>adenovirus 2</u> genomes contained in <u>Ad2-SV40 hybrid virus DNA molecules</u> . The hybrid viruses are also used as models for understanding the behavior of <u>integrated genomes</u> . We are also analyzing the behavior of SV40 and <u>Ad2-transformed</u> or <u>lytically</u> infected cells as a function of the <u>molecular conformation of viral nucleic acids</u> within these cells, and as a function of events which may control (or be controlled by) <u>viral replication, transcription, and translation</u> . Secondly, we are analyzing the state and expression of the endogenous <u>AKR murine leukemia virus genome</u> in mice with varying expression of <u>leukemia and virus production</u> , as well as exploring the <u>evolution, transmission, and relation to leukemogenesis</u> of various murine RNA viruses. The techniques of <u>restriction endonuclease mapping</u> and <u>molecular cloning</u> are being applied to this genetic analysis of <u>ecotropic, xenotropic, amphotropic</u> and <u>recombinant</u> murine leukemia viruses.		

Objectives:

1. To study the genetic organization and expression of Simian Virus 40 (SV40) and human Adenovirus 2 (Ad2) by an analysis of the varying segments of the SV40 and Ad2 genomes contained within Ad2-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, biophysical, and electron microscopic techniques to achieve genetic maps of these animal tumor viruses. The hybrid viruses are also employed as models for understanding the behavior of integrated genomes, the phenomenon of integration appearing central to the molecular mechanism of viral oncogenesis.
2. To analyze the behavior of SV40 and Ad2-transformed or lytically infected cells as a function of the molecular conformation of viral nucleic acids within these cells, and as a function of events which may control (or be controlled by) viral replication, transcription, translation and maturation. The early antigens of SV40 and their role in cell transformation and tumor rejection are of particular interest.
3. To enhance our understanding of the etiology and natural history of murine leukemia by biochemically and genetically analyzing various murine leukemia viruses, both those occurring naturally as well as laboratory-derived strains. We believe that such analyses will not only improve our understanding regarding leukemia-causing viruses, but also our understanding regarding the nature of high-, low-, and non-leukemic strains of mice. A further objective is to explore the evolution and transmission of these viruses, using cells from a variety of wild rodents and related species, and using comparative viral genetic maps derived through the use of restriction endonucleases and molecular cloning of viral nucleotide sequences.

Methods Employed:

Procedures for the isolation of highly purified viral and cell macromolecules are continuously improved and new techniques for hybridization of viral and cellular nucleic acids are developed or adapted. A variety of techniques which permit quantitative comparison of nucleotide sequences are in use. This laboratory routinely employs equilibrium density centrifugation, analytical ultracentrifugation, electron microscopic heteroduplex mapping, enzymatic fragmentation and strand separation of viral DNA, gel electrophoresis, hydroxyapatite chromatography, and the in vitro synthesis of viral DNA probes. Nucleotide sequencing of specific viral RNA transcripts is available. In vitro protein synthesis using cell-free systems is undertaken. An extensive virus purification and tissue culture facility is maintained; plaque assays and CF or IF tests for viral-specific antigens are performed. Radiolabelled proteins are analyzed via autoradiography or fluorography; fingerprint analysis of the tryptic peptides of purified proteins is performed by a two-dimensional procedure involving high-voltage

electrophoresis and thin-layer chromatography. Mouse breeding experiments are performed in association with studies on the genetics of RNA tumor viruses. Viral DNA sequences, either in pure form or integrated into the cellular DNA, have routinely been molecularly cloned using bacterial or plasmid-vector systems. Restriction endonuclease mapping of unintegrated, integrated or molecularly cloned viral DNAs is performed. New cell lines which can support better yields of virus or can be utilized as better assay systems are developed continuously. New viruses are also isolated and characterized both biologically and biochemically. These developments specifically involve single cell cloning, fluorescent antibody assay techniques, virus titration, reverse transcriptase assays, sucrose and caesium density gradient purification, and Southern blotting techniques, in addition to the methods described above.

Major Findings:

A. Studies on the Murine Leukemia Viruses

1. Restriction endonuclease mapping of ecotropic viral DNAs: Size and sequence heterogeneity of the terminal repeat sequence - A physical map using 12 restriction endonucleases has been derived for the unintegrated linear viral DNA of 11B3 murine leukemia virus (MuLV), which is the endogenous ecotropic XC plaque forming virus released by the cell line from which integrated MuLV molecular clones 614 and 623 were isolated. This virus had originally been isolated from a mouse carrying the Akv-1 locus. The 11B3 MuLV DNA map was compared with the maps derived for the DNAs of several other endogenous ecotropic MuLVs isolated from inbred mouse strains and from the Asian feral mouse *Mus musculus mollosinus*. In general, their maps were highly related, indicating a common origin for most of their sequences. However, 11B3 MuLV DNA differed from the other MuLV DNAs in two important respects: Its terminal repeat sequence was about 50 bp longer than that of the other MuLVs and it lacked a Hind III site which was located 3 kbp from the 5' end in the other MuLVs. The results suggested that these two differences in 11B3 MuLV had arisen after virus activation in the mouse, probably by recombination with endogenous sequences of xenotropic MuLV origin. In the endogenous ecotropic MuLV DNAs (including 11B3 MuLV), the region of the terminal repeat sequence which is derived for the 3' end of the viral RNA differed from this region in Moloney MuLV for each of the 5 restriction endonuclease sites which were tested. We conclude that the terminal repeat of murine C-type viruses may contain size and sequence heterogeneity and that recombination with endogenous viral sequences may occur in the terminal repeat and in the 5' portion of the virus.

After establishing the restriction endonuclease map for 11B3 MuLV DNA, we analyzed several other endogenous ecotropic MuLVs using the

same enzymes. These enzymes include: EcoRI, Hind III, Xho I, KpnI, SmaI, PvuII, Pst I, Sal I, Sac I, Cla I, Bam HI, and Xba I. The ecotropic viruses that we analyzed were another AKV-1 isolate (MB 427), two AKV-2 isolates (MB 358, MB 359) and one isolate each from BALB/c, C58, C3H/HeN, C3H/Fg, and Kyushu mouse (Mus musculus molossinus). With the exception of C3H/Fg, the restriction endonuclease maps of all of the ecotropic viruses tested are identical. None of these ecotropic viruses has an EcoRI restriction endonuclease recognition site, and all of them have two sites for Pvu II recognition. However, the ecotropic virus from C3H/Fg has one recognition site each for EcoRI and Pvu II.

2. Molecular cloning of infectious integrated murine leukemia virus DNA from infected mouse cells - The lack of an endonuclease EcoRI site in the AKR murine leukemia virus (MuLV) DNA genome was utilized to molecularly clone, in Charon 4A λ DNA, integrated infectious AKR MuLV DNA isolated from productively infected mouse cells. Three λ -mouse recombinants (clones 614, 621, and 623) were selected by virtue of their reactivity with AKR MuLV [^{32} P]cDNA. Clones 614 and 623 contained the complete AKR MuLV DNA flanked by nonviral cell sequences of which no more than 100 base pairs beyond the viral DNA appear to be shared. DNAs from both clones 614 and 623 were highly infectious for mouse cells and yielded N-tropic ecotropic MuLV; the specific infectivity of the DNA and the titer of the derived virus was more than 10-fold higher with 623. Clone 621 contained only some viral DNA and was not infectious under similar conditions.
3. Close similarity between endogenous ecotropic virus of Mus musculus molossinus (Kyushu mouse) and AKR - To date, ecotropic Gross AKR-type virus has been isolated only from certain inbred strains of mice and not from wild Mus musculus of various geographic origins. By using nucleic acid hybridization techniques, we have demonstrated that the complete genome for AKR type virus is present in some inbred mice (e.g., AKR, C58, C3H/Fg, PL, BALB/c, DBA/2, C3H/HeN, C57BR, C57BL, etc.), but that only an incomplete genome (about 80%) of AKR related sequences is present in other inbred strains (e.g., NIH Swiss, C57L, 129, wild Mus musculus from Maryland, California and West Bengal, India). This result poses an important question regarding the origin of AKR virus.

To determine whether AKR is truly a naturally occurring virus, we examined DNAs from various rodents for the presence of AKR viral sequences. In addition to various wild Mus musculus from different geographic regions, the list of rodents includes: Mus cervicolor, M. cookii, M. dunii, M. pahari, M. musculus castaneus, and M. m. molossinus. Of these animals, only DNAs from M. m. castaneus and M. m. molossinus completely saturated the AKR [^{32} P]cDNA probe, indicating that the complete genome of AKR type virus is present in

these two Asiatic mus. We isolated both ecotropic and xenotropic viruses from M. m. molossinus and M. m. castaneus, and analyzed the ecotropic virus of M. m. molossinus quite extensively both biologically and biochemically.

M. m. molossinus is a high-virus mouse in that murine leukemia viruses can be obtained readily from the tissues of young adult mice and can be easily activated from tissue culture cells using 5-iododeoxyuridine. In its biological behavior, the molossinus ecotropic virus is like the endogenous ecotropic strains of laboratory mouse origin. That is, this virus is N-tropic, ecotropic and XC⁺. On biochemical analysis, it was also indistinguishable from AKR virus. First, RNA from purified molossinus virus saturated 100% of the sequences of an AKR virus [³H]-cDNA probe. Second, restriction endonuclease analysis with twelve enzymes yielded identical genomic maps for both AKR virus and molossinus virus. Finally, an AKR [³H]DNA probe can be completely saturated with molossinus DNA and the DNA hybrid molecules are very well matched as determined by T_m analysis. The only difference that we could find between AKR DNA and molossinus DNA with respect to AKR viral sequences has to do with the way in which these sequences are arranged in the host cell DNA. However, the isolation of an identical virus from the Japanese wild mouse Mus musculus molossinus leaves no doubt that AKR-type virus is present in nature.

4. Biochemical analysis of amphotropic and recombinant viruses - Recently, considerable attention has been given to the MCF (Mink Cell focus-forming) type of recombinant virus since this class can produce a high leukemia incidence in non-leukemic strains of mice. By host range, the MCF class of viruses is amphotropic in nature. On the other hand, several laboratory strains which are ecotropic in their host range can produce an even higher incidence of leukemia than the MCF strains. The origin and nature of these laboratory-derived viruses remain obscure, and numerous attempts to re-isolate such viruses from inbred strains have failed. By using a purified "ecotropic-specific" probe (the portion of AKR viral sequences which does not cross-react with xenotropic and amphotropic viruses), and an "amphotropic-specific" probe, we have been able to demonstrate that laboratory-derived strains, e.g., Moloney and Rauscher, are also recombinant strains involving amphotropic-specific sequences. On the other hand, MCF viruses, isolated from pre-leukemic or leukemic mice, are recombinants between AKR-ecotropic virus and a xenotropic virus and thus are phenotypically amphotropic. True amphotrophic virus, although possessing a dual-tropic host range, is not a recombinant between any of the ecotropic and xenotropic viruses of laboratory mice thus far isolated. Thus in nature, there are at least two sets of sequences, either one of which can specify the ability of a virus

to grow in mouse cells. Detailed genetic mapping of these sequences is in progress.

5. Regulation of endogenous Type C viruses: Evidence for transcriptional control of AKR viral expression - The expression of the AKR C-type RNA tumor virus has been studied in cell lines derived from AKR mouse embryos. Although all AKR cell lines examined contain the proviral sequences integrated into their genomes, viral producer and non-producer lines have been identified. Using a ^3H -labeled DNA complementary to AKR viral RNA sequences, the presence of viral RNA sequences in the nucleus and cytoplasm of viral producer cells has been detected. Chromatin from viral producer cells is an effective template for transcription of AKR viral RNA sequences. A significantly reduced level of hybridization is observed between AKR viral cDNA and nuclear RNA, cytoplasmic RNA, and in vitro chromatin transcripts from non-producer AKR cells. Regulation of viral gene expression mediated at least in part at the transcriptional level is therefore suggested. The low level of hybridization of viral cDNA with in vivo and in vitro transcribed RNAs from non-producer cells may reflect either partial AKR viral transcripts or other endogenous, closely related C-type viruses. Although chromatins of viral producer and non-producer cells differ in their ability to transcribe AKR viral sequences, high resolution two-dimensional analysis of chromosomal proteins from both cell types does not reveal significant differences.

B. A Quantitative In Vitro Focus Assay for Bovine Papilloma Virus

A quantitative tissue culture assay for bovine papilloma virus (BPV) has been developed. Extracts of bovine papillomas which contained either BPV-1 or BPV-2 induced distinct foci in two mouse cell lines (NIH 3T3 and C127). Focus formation followed single-hit kinetics, suggesting that a single particle was required for focus induction. Preincubation of extracts with rabbit anti-BPV-1 serum neutralized their infectivity; the neutralizing activity of anti-serum was greater for a BPV-1 extract (1:16,384) than for a BPV-2 extract (1:2048). The morphologically transformed cells contained BPV DNA sequences, grew in 30% fetal calf serum and in soft agar, and were tumorigenic for nude mice. BPV-2 extracts did not induce distinct morphologic alterations in human, rat, and bovine conjunctival cells tested.

C. Molecular basis of increased synthesis of albumin in rat liver after thioacetamide administration

Administration of thioacetamide to rats for 4 days increases the activity of liver mRNA and disproportionately increases the activity of albumin mRNA in the wheat germ translation system. We have explored the possibility that administration of thioacetamide quantitatively

alters the transcription of rat liver unique DNA. Nucleic acid hybridization between radioactive rat unique DNA and RNA from untreated, as well as 4-day thioacetamide treated, rat liver indicates that the normal extent of transcription is not altered by the drug treatment. We also investigated the possibility that thioacetamide treatment increases the level of mRNA in general and specifically of albumin mRNA. Albumin mRNA is the most abundantly represented population in rat liver cytoplasmic mRNA and was further enriched by sucrose gradient fractionation of the liver mRNA. Synthesis of cDNA and then use of the strategy of limited hybridization yielded the cDNA corresponding to albumin mRNA. Hybridization of the fractionated cDNA with mRNA from untreated, as well as from livers with increasing days of treatment, shows no change in the level of albumin mRNA and indicates that drug treatment causes the disproportionately increased synthesis of albumin by disproportionately increasing the translational activity of albumin mRNA. Quantitation of gene dosage by comparison of hybridization kinetics of fractionated cDNA and unique [^3H]DNA with total DNA indicated that there are two copies of the albumin gene present in the rat genome.

D. Inhibition of Papovavirus DNA Synthesis by Superinfection with Ad2

It is known that SV40 DNA synthesis is inhibited in monkey cells by superinfection with Ad2. The inhibition appears to be mediated by an early Ad protein or by a host protein induced early in Ad infection. During the past year we have completed a study of the kinetics of inhibition of SV40 and BKV DNA synthesis in monkey and human cells superinfected with Ad2 and Ad2-SV40 hybrid viruses. The results of this study are summarized as follows: 1) A kinetic analysis shows that inhibition begins early in the Ad2 lytic cycle. This result is in agreement with previous studies using temperature sensitive mutants of Ad5. 2) Inhibition is less efficient when superinfection is carried out late in the SV40 lytic cycle when compared to superinfection early. Moreover, the onset of Ad2 DNA synthesis is delayed when superinfection is effected after SV40 DNA synthesis is underway. These observations suggest that there may be competition between replication SV40 and Ad2 genomes to bind factors which are inhibitory for SV40 DNA synthesis and are essential for Ad2 DNA synthesis. 3) The kinetics of inhibition of BKV DNA synthesis in human cells are similar to those of inhibition of SV40 DNA synthesis in monkey cells, suggesting that host factors may not be involved in the inhibition process. 4) The Ad2-SV40 hybrid viruses Ad2⁺ND₃, Ad2⁺ND₄, and Ad2⁺ND₅ are efficient inhibitors of SV40 DNA synthesis. This finding is of interest since the early Ad2 E3 template is largely deleted from these viruses; thus, the proteins encoded in E3 are not the inhibitory factors. 5) When all DNA synthesis is temporarily blocked by hydroxyurea, inhibition is about the same as in the absence of hydroxyurea although Ad2 DNA synthesis is reduced 5- to 10-fold. This result indicates that Ad2 DNA synthesis per se is not required for

inhibition and obviates the possibility that inhibition is a consequence of binding of factors essential for SV40 DNA synthesis by replicating Ad2 genomes. Moreover, this result supports the notion that inhibition is an early Ad2 function. 6) Super-infection with Ad2 does not alter the accumulation of SV40 T antigen in dually infected cells indicating that the mechanism of inhibition is not mediated by blocking synthesis of initiators of SV40 DNA synthesis.

Together these results suggest that an early Ad2 protein which is essential for Ad2 DNA synthesis binds to SV40 DNA and either blocks initiation of SV40 DNA synthesis by interfering with binding of SV40 T antigen or, alternatively, by blocking chain elongation. These results may indicate that the early 72K Ad2 DNA binding protein is involved in inhibition of SV40 DNA synthesis; this protein is required for Ad2 DNA synthesis.

E. Oncogenicity of Somatic Cell Hybrids Formed from SV40- and Ad2-Transformed Hamster Cells

The interactions between Ad and SV40 in lytic infection appear to involve functions of early viral proteins. The 90K SV40 protein enhances Ad replication in monkey cells and an early Ad2 protein (possibly the 72K DNA binding protein) suppresses SV40 DNA synthesis in coinfecting monkey cells. Since transformation by SV40 and Ad are also functions of early viral proteins, it would be of interest to determine whether expression of both viral genomes in the same transformed cell would influence the oncogenic potential of the hybrid transformed cell.

A. M. Lewis, Jr., has established a number of lines of hamster cells transformed by either Ad2 or by SV40. These cell lines have been characterized with respect to their oncogenicity in immunocompetent (adult) and immunoincompetent (newborn) syngeneic (LSH) hamsters and in adult allogeneic (CB) hamsters. The Ad2-transformed cell lines induce tumors only in newborn LSH hamsters, while the SV40-transformed cell lines readily induce tumors in adult LSH and CB hamsters with a TPD₅₀ of $10^3 - 10^4$ cells (TPD₅₀ = dose or number of cells required to produce tumors in 50% of the animals inoculated s.c.). In collaboration with Dr. Lewis, we have established transformed hamster cell lines which lack either thymidine kinase (TK⁻ mutants will propagate in media containing BrdUrd) or hypoxanthine guanine phosphoribosyl transferase (HPRT⁻ mutants will propagate in media containing 6-thioguanine). Neither TK⁻ or HPRT⁻ mutant cell lines will propagate in media containing hypoxanthine, aminopterin, and thymidine (HAT media). The mutant cell lines have been cloned and a variety of clones of Ad2-transformed (HPRT⁻) cells have been hybridized with SV40-transformed (TK⁻) cells by treatment with polyethylene glycol and selection in HAT medium. Hybrid cell lines have been cloned and characterized with respect to morphology (Ad2-like or SV40-like), chromosome index (diploid or tetraploid), and viral T

TABLE I

Tumor incidence of mutant Ad2- and SV40-transformed hamster cells and of somatic cell hybrids in syngeneic (LSH) and allogeneic (CB) Hamsters. (a)

CELL LINE	CELL TYPE	MORPHOL- OGY	ANTIGEN SV40 Ad2	NEW BORN		ADULT		ADULT	
				LSH HAMSTER TIME (WEEKS)	TUMOR (b) INCIDENCE	LSH HAMSTER TIME (WEEKS)	TUMOR INCIDENCE	CB HAMSTER TIME (WEEKS)	TUMOR INCIDENCE
PH1	SV40 HE2(TK-)	SV40	+	-	6	18/18	11 (4/5)(c)	11	2/5
PH2	Ad2 HE3(HPRT-)	Ad2	-	+	6	16/16	11	0/5	0/5
PH3	Ad2 HE3xSV40HE2	Ad2	+	+	2	20/20	10	3/4	0/4
PH4	Ad2 HE3xSV40HE2	SV40	+	+	10	10/15	9	0/4	0/4
PH5	Ad2 HE3(HPRT-)	Ad2	-	+	3	13/13	8	0/4	0/4

a) The data shown were obtained by subcutaneous injection of 0.2ml of cell suspension containing 10^7 cells.

b) Tumor Incidence = number of animals with tumors/number of animals inoculated.

c) This result was obtained with an inoculum of 10^4 cells.

antigens (Ad2 or SV40). We are now engaged in determining the oncogenic potential of these mutant and hybrid cell lines in newborn and adult LSH and adult CB hamsters. The first test is summarized in Table 1. Although this test is incomplete at the present time (it may require six months to reach a definitive answer), several points may be made. First, the oncogenicity of the mutant SV40 HE2 (TK-) appears to be attenuated with respect to the nonmutant parent cell line. The mutant cell line induces tumors in adult syngeneic hamsters with about the same TRD₅₀ as the nonmutant parent although the latent period for tumor formation by the mutant is much longer. The significance of the duration of the latent period is unclear but may reflect the number of oncogenic cells in the inoculum. Although the mutant cell line induces tumors in adult allogeneic hamsters, the TPD₅₀ is 10³ to 10⁴ times larger than the TPD₅₀ for nonmutant SV40-transformed cells. Secondly, the oncogenic potentials of the hybrid cell lines are even less than that of the nonhybrid SV40-transformed mutant from which they were derived. One line (PH 3) induces tumors in adult syngeneic hamsters but the TPD₅₀ is 10³ to 10⁴ times larger than for SV40 HE 2 (TK-); this line has induced no tumors in adult allogeneic hamsters after ten weeks. The other hybrid line tested (PH 4) has induced no tumors in either adult syngeneic or allogeneic hamsters; its ability to induce tumors in newborn syngeneic hamsters is even less than that of the Ad2 HE3 (HPRT-) parent from which it was derived.

The reduced oncogenicity of the mutant (TK-) SV40-transformed cell line was unexpected and may reflect a loss of macrophage resistance characteristic of the nonmutant parent. This possibility is currently being tested in collaboration with Dr. J. L. Cook who has developed an in vitro assay to determine the ability of macrophages to recognize and destroy cells of syngeneic or allogeneic origin. In addition, it will be of interest to determine whether SV40-transformed (HPRT-) mutants also exhibit an altered oncogenic potential. Accordingly, we will compare in a single test, the oncogenic potentials of both mutant SV40-transformed cell lines (TK- and HPRT-) with the nonmutant parent cell line from which they were derived. If this apparent attenuation of the oncogenicity of SV40-transformed cells is authentic, it will be of interest to determine whether and/or how the enzymes involved contribute to the oncogenicity of these cells. These cell lines will be further characterized with respect to the content of viral DNA and the expression of viral DNA. In addition, we will determine whether the integrated virus can be "rescued" from the mutant cell lines. Similarly, the apparent loss of SV40-like oncogenicity in hybrid cell lines which contain both TK and HPRT is also of interest. The tumors induced by these lines will be examined histomorphologically. In addition, the hybrid cell lines and the tumor lines established in vivo will be characterized with respect to viral DNA content and viral genetic expression.

To summarize, the results of our first test indicate that the oncogenicity of SV40-transformed cells is attenuated by simply selecting TK⁻ mutants from a population of transformed cells. The oncogenicity of the mutant cell lines is further attenuated by hybridizing them with Ad2-transformed cells. No clear pattern of oncogenic dominance in hybrid transformed cells has emerged since one hybrid (PH 4) is evidently less oncogenic than the mutant (HPRT⁻) Ad2-transformed cell line from which it was derived, while the other hybrid (PH3) is somewhat more oncogenic for adult LSH hamsters than is the parent mutant. Some hybrid cell lines that have been established appear to lack Ad T antigen while retaining the ability to express SV40 T antigen. It will be of interest to determine whether these cell lines too exhibit modified oncogenicity.

Significance to Biomedical Research and the Program of the Institute:

In studies on the mouse-tropic, xenotropic, and amphotropic murine leukemia viruses, our objective is to correlate specific leukemia virus sequences with specific biological events, especially leukemogenesis. Comparative study of the genetic information specified by different murine leukemia virus sequences in different strains of mice is now feasible, and we believe that it will be possible to determine the evolutionary origin and transmission of murine leukemia virus infection. Our studies have explained why only some mice produce virus and develop leukemia even though all mice may contain MuLV antigens. Moreover, it has been established that a major mouse chromosomal locus for the production of MuLV is in fact composed of MuLV nucleotide sequences, and is not merely a host locus which permits the expression of remote viral functions. Finally, our recent studies suggest that recombination between the genomes of various naturally occurring murine viruses may be the necessary and sufficient event which eventuates in leukemogenicity. This and other hypotheses can now be tested quite directly via the manipulations made possible by molecular cloning. In this research, we have recently succeeded in molecular cloning of the first murine leukemia virus DNA known to be integrated and infectious. It should be emphasized that these studies concern known and controlled host genetics. We believe that these studies will guide the framing of testable hypotheses in human leukemia, wherein a proven leukemia virus has not been characterized and where the genetics of the disease are not known and cannot be controlled.

Our studies of Ad2-SV40 hybrids have made a significant contribution to knowledge of genetic organization and expression of the two tumor viruses during lytic infection and oncogenesis. Moreover, these hybrid virus studies offer insight into the state and expression of integrated heterologous DNA in general, and must be considered in planning the type of genetic manipulations possible with recombinant DNA technology.

Whether a specific viral infection will lead to virus production or to cell transformation depends entirely upon the interaction between the incoming

virus and the host cell involved. At the molecular level, such interactions may occur between virus-specific macromolecules and macromolecules specified by the host, and similar interactions may occur between the products of two viral genomes (e.g., "enhancement"). Our studies with Ad2 and SV40 are directed toward building a model of these interactions; our hope is to provide a better understanding of the mechanisms of viral infection by examining the effects these two genomes have on each other during co-infection. Similarly, our studies of somatic cell hybrids derived from SV40- and Ad2-transformed parent cell lines may indicate how interactions between these two viral genomes influence oncogenicity. Finally, the presence of tumor-specific transplantation antigens in viral induced tumor cells may permit immune modulation of the growth of antigenic tumors; thus the biochemical characterization of such macromolecules is of potential importance. Our ability to assign the tumor rejection activity induced by SV40 to a specific polypeptide chain (28K) should permit study of the molecular mechanism of action of a single macromolecule in this regard.

Proposed Course:

We shall continue to employ various biochemical and biological techniques (including molecular cloning of MuLV sequences utilizing recombinant DNA technology) to distinguish between the viral sequences specified by different endogenous murine leukemia viruses in the DNA of mice with varying expression of MuLV information. DNA from other rodent species will be tested for homology with various MuLV DNA probes. Collectively, these studies should speak to the evolutionary origin, transmission, completeness and expression of the MuLV genome, as well as to the recombinational events displayed by various endogenous viruses and the generation of highly leukemogenic viruses.

With regard to current research on inhibition of SV40 DNA synthesis by Ad2, an in vitro SV40 DNA synthesising system has been described by M. DePamphilis and his colleagues. This system utilizes nuclei containing radiolabeled SV40 replicative forms together with cytoplasmic extracts from uninfected, or SV40 infected cells, and a mixture of DNA precursors (i.e. deoxyribonucleotide triphosphates). Under proper conditions, SV40 replicative forms (initiated in vivo) can be converted to mature SV40 superhelical DNA in vitro. We propose to determine whether conversion is blocked by cytoplasmic extracts from Ad2 infected cells; if so, it will indicate that Ad inhibition acts at the level of chain elongation. If a cytoplasmic extract containing early Ad2 proteins does not inhibit, it may suggest that Ad inhibition acts at the level of replication initiation, although a negative result would not preclude other possibilities. If inhibition can be demonstrated in this system, it should be possible to identify the specific proteins involved. For example, the 72K Ad2 DNA binding protein can be specifically removed by treating the cytoplasmic extracts with single-stranded DNA. Alternatively, specific purified Ad2

proteins can be added to the system to determine their effect on SV40 DNA synthesis.

We have recently initiated a new set of studies concerned with the molecular mechanisms of action displayed by interferon. It is known that SV40 T antigen synthesis in SV40 infected monkey cells is almost completely suppressed by treatment with interferon (30-100 units/ml). The mechanism of inhibition appears to reside at the level of mRNA translation although in cells pretreated with interferon there is an 80-90% reduction in the level of early viral mRNA. If SV40 infected cells are treated with interferon late in infection, there appears to be no diminution of the amount of viral mRNA although T antigen synthesis is inhibited. One mechanism which might account for the observed action of interferon on SV40 T antigen synthesis involves the possible alteration of the splicing pattern of the viral mRNA. This possibility has not previously been investigated, although it could account for many of the observed effects of interferon. Specifically, we propose to prepare highly radioactive RNA from SV40 infected cells and to hybridize this RNA with DNA fragments, prepared by restriction endonuclease digestion of SV40 DNA immobilized on nitrocellulose filters. A comparison of the hybridization pattern of SV40 RNA prepared from untreated cells with that prepared from cells pretreated with interferon should indicate whether or not the splicing pattern of viral mRNA is significantly altered by interferon.

Finally, the question of the influence of interferon on tumor rejection will be examined. A correlation exists between the resistance of transformed cells to macrophage lysis in vitro and the ability of the cells to induce tumors in allogeneic animals (J.L. Cook, J.B. Hibbs, Jr., and A.M. Lewis, Jr., submitted for publication). Thus in cells transformed by SV40 (which are insensitive to macrophage lysis) the cell surface is modified in such a way as to prevent macrophage lysis both in vitro and in vivo. It would be of interest to determine whether the macrophage sensitivity and the oncogenicity of SV40-transformed cells is modified by culturing cells in vitro in the presence of hamster interferon. In addition, it would be of interest to determine whether the oncogenicity of these cells is modified when inoculated into animals which have been treated with interferon or with inducers of interferon (i.e., poly I - poly C). Similarly, Ad2-transformed cells which have been "conditioned" to induce tumors in adult LSH hamsters, and Ad2-transformed + SV40-transformed cell hybrids which induce tumors in syngeneic hosts (Table 1), could also be examined in vitro and in vivo to determine whether interferon modifies the macrophage sensitivity or oncogenicity of these cells. Such experiments could provide insight into the mechanism by which interferon enhances the ability of a given host to reject an oncogenic challenge.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06860-04 PO
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Clinical and Molecular Pharmacology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: D. L. Glaubiger OTHER: L. Cohen D. G. Poplack K. Kohn A. Talaizadeh A. Ramu N. P. Ramu J. Holcenberg W. A. Bleyer D. Gangji K. Kraemer	Senior Investigator Clinical Associate Senior Investigator Chief Visiting Fellow Visiting Scientist Visiting Fellow Professor Assoc. Prof. Guest Worker Senior Investigator	PO C PO C PO C LMPH C PO C PO C PO C Med. College Wisc. U. of Washington PO C CB C
COOPERATING UNITS (if any) Med. College of Wisconsin, U. of Washington, Seattle; Laboratory of Molecular Pharmacology, NCI; Clinical Pharmacology Branch, NCI.		
LAB/BRANCH Pediatric Oncology Branch		
SECTION -----		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 8	PROFESSIONAL: 6	OTHER: 2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
This project concerns clinical and pre-clinical studies of anti-cancer drugs. Using a previously developed <u>assay for citrovorum factor</u> and <u>5'methyltetrahydrofolate</u>, the distribution of these compounds in plasma and cerebrospinal fluid in man and in primates has been elucidated. The mechanism of methotrexate excretion from CSF in man has been further explored. <u>In vitro</u> evaluation of the relative cytotoxicity of methotrexate and methotrexate in the presence of probenecid has been performed. <u>DNA damage and repair</u> following exposure to <u>intercalating agents</u> including <u>light-sensitive compounds (psoralens)</u> have been further explored. Characteristics of <u>drug-DNA complexes</u> for both psoralen derivatives and for <u>diacridines</u> have been measured. An <u>in vitro tumor cell cloning technique</u> has been explored with regard to the growth of various kinds of tumors and the excretion of known <u>tumor cell markers</u>.		

Objectives:

1. To develop and apply clinically useful assay systems for chemotherapeutic agents.
2. To characterize the pharmacokinetics and distribution of chemotherapeutic agents and related compounds to enhance their clinical utility.
3. To investigate mechanisms of sensitivity and resistance of tumor cells to chemotherapeutic agents.
4. To utilize in vitro biochemical and biophysical measurements to characterize interactions of chemotherapeutic agents with whole cells and subcellular components, and to examine biochemically the effects of such interactions with regard to damage and repair of subcellular components.

Major Findings and Methods:A. Clinical Studies

1. Methotrexate pharmacokinetics - We have studied the cerebrospinal fluid pharmacokinetics of methotrexate in man, and utilizing previous results obtained in animals with probenecid and a bulk flow marker, indium-labeled DTPA, we were able to demonstrate that CSF efflux in man has both active and passive components. Our observations to date, however, do not fully explain the relationship of clearance of CSF methotrexate to bulk flow (methotrexate appears to be cleared from CSF at about one-half of the rate of bulk flow). We have also utilized an assay for citrovorum factor in evaluating both plasma and CSF pharmacokinetics of both citrovorum factor and its major metabolite, N⁵ methyltetrahydrofolate. Results indicate that in man, as in previously described animal studies, the plasma half-life of citrovorum factor is short and the half life of N⁵ methyltetrahydrofolate is longer. After an injection of citrovorum factor, equimolar concentrations of N⁵ methyltetrahydrofolate and citrovorum factor are found at approximately 40 minutes, after which N⁵ methyltetrahydrofolate becomes the major folate derivative in plasma. With regard to penetrance into the CSF, we had previously shown in animals that leucovorin penetrated the CSF with approximately the same efficiency as methotrexate (30% of the concomitant plasma level). N⁵ methyltetrahydrofolate, on the other hand, is far more efficient in penetrating the blood-brain barrier and equimolar concentrations are found in both CSF and plasma after approximately 1 hour.

2. Platinum studies - We have begun to evaluate the possibilities of measuring platinum in body fluids using atomic absorption spectroscopy. Preliminary results indicate that we can measure elemental platinum in both plasma and cerebrospinal fluid with relative ease and, in one patient with CNS toxicity from platinum administered intravenously, we were able to detect residual platinum in plasma. Concomitant CSF platinum levels were below the level of detection ($10^{-7}M$). However, the difficulty with these measurements is that elemental platinum does not, in fact, reflect active drug and therefore the significance of measurements indicating residual elemental platinum in body fluids is unclear.

B. In Vitro Studies

1. Methotrexate and probenecid - We have previously shown, using an in vitro soft agar cloning technique, that probenecid inhibits methotrexate cytotoxicity in mouse L1210 leukemia cells, that this effect is dose-related, and that it does not appear to be due to an effect of probenecid on penetrance of methotrexate into cells. Intracellular methotrexate levels, in the presence and absence of probenecid, are equal. There does, however, seem to be some effect of probenecid on the cell cycle, as reflected in the growth kinetics of cells continuously exposed to probenecid. The significance of this effect remains to be elucidated.

Previous measurements related to concentration x time cytotoxicity of methotrexate in L1210 cells have been repeated using human Burkitt's lymphoma cells, again using a soft agar cloning technique as a measurement of cytotoxicity. The results with the L1210 cells had indicated that above a threshold concentration, the main determinant of methotrexate cytotoxicity was the time of drug exposure and not the concentration achieved. Similar results are obtained using human Burkitt's lymphoma cells. However, the effect is smaller than that seen with L1210. We are presently repeating these results to examine the effect of the technique by which the soft agar cloning is carried out on the magnitude of the results, with particular regard to the human cell.

2. Human tumor stem cell assay -The human tumor stem cell assay, developed by Hamburger and Salmon, was initially used in this laboratory to screen a large number of both adult and pediatric malignancies for their ability to grow in this system, as well as the possible utility of such a system for screening drugs against individual human tumors of various types and also for possibly screening new agents. It became apparent that there were certain problems associated with this system: 1) Not all tumors grow in the system - approximately 70% of those attempted will grow; 2) the plating efficiency of most tumors is extremely low when attempted from patient material; 3) the method by which the cells

should be exposed to antineoplastic agents for an in vitro screen such as this is unclear. The present method of drug treatment in vitro is to expose the cells in suspension for 1 hour prior to plating or to continuously expose the cells to drug in the agar plate. We have attempted to solve some of these problems, in particular the low plating efficiency and the method of drug exposure, using as a model human neuroblastoma cell lines. The decision to use cell lines rather than primary explants from patients was made because of the requirement, in such a set of experiments, for a reproducible source of cells. It had also been shown previously that freezing and thawing of cells affected their ability to grow in the stem cell assay.

Results to date have shown that plating efficiencies can be improved from approximately 1-40% using primary explants of human tumors to approximately 35% by subculturing the cell lines. One of the factors associated with this improvement appears to be the point in the growth curve of the cells when they are taken for plating. If the cells are taken during the lag phase, plating efficiency is extremely low. As one proceeds up the lag phase curve towards the plateau phase, the plating efficiency improves. Repetitive sampling near the plateau phase, using several serial subcultures of the cells, continuously improves the plating efficiency up to about 35%. The implication of these data is that the low plating efficiency associated with the stem cell assay as applied to patients may be related to the portion of the growth curve from which the cells are taken. With high plating efficiencies we have been able to obtain dose-response curves to chemotherapeutic agents using the human tumor stem cell assay for human neuroblastoma lines. These dose-response curves for adriamycin resemble those obtained for L1210 or other mouse cells when exposed to adriamycin. We are also attempting to use hyperthermia for protracted periods of time to obtain a dose-response curve for the human neuroblastoma cells. Preliminary results indicate that response curves can be obtained which are similar to drug dose-response curves.

We have also begun, in collaboration with Dr. Kurt Kohn of the Laboratory of Molecular Pharmacology, to investigate the utility of this system in evaluating newer nitrosourea compounds, particularly chloroethylnitrosourea (CNU) which has high alkylating activity but little carbamoylating activity. We plan to compare the CNU with either CCNU or BCNU in vitro using the human tumor stem cell assay and, if CNU appears to be more active as an antitumor agent, we will then carry the same screening procedures forward to primary explants from patients, looking for the same effect. The ultimate objective of this line of experiments would be to support the

notion that nitrosoareas currently being developed, which have relatively high alkylating activity, would be more effective anticancer drugs than those now in use.

3. Adriamycin resistance - We are also investigating mechanisms of resistance in an *in vivo* adriamycin-resistant P388 cell line. To date, our studies indicate that the cell line which is more sensitive to adriamycin is, in fact, more resistant to x-irradiation. The mechanism for this is unclear. Catalase levels in the relatively x-ray resistant line are higher than in the more x-ray sensitive line. Superoxide dismutase levels are comparable in both lines. We are presently investigating glutathione reductase levels in both lines (which seem to be equal in preliminary studies), and measuring DNA damage and repair in both cell lines using the alkaline elution technique described by Kohn and Wig (Biochemistry, 1971).
4. Psoralens - We have been studying psoralens (light-sensitive bifunctional alkylating agents) in L1210 mouse leukemia cells and have shown that, in whole cells, these agents reproduce the quantitative relationships (with respect to light exposure and alkylating activity) that have been observed *in vitro* using purified DNA systems. In pursuing these studies further, we have evaluated the effect of light in the absence of psoralen on these cells and found that, although there is no effect of light at 365 nm (the wavelength at which psoralens are irradiated for activation), at lower wavelengths significant numbers of crosslinks are induced as measured by the alkaline elution assay. We have also studied angelicin, a psoralen derivative which is incapable of forming bifunctional crosslinks but is able to form monoadducts, and have found that these monoadducts yield no detectable crosslinks in the alkaline elution assay. This is an important control experiment for the others which have been performed. Quantitative relationships between psoralen-induced crosslinking and cytotoxicity have been demonstrated in human lymphocytes in collaboration with Dr. Kenneth Kraemer of the Laboratory of Cancer Biology.
5. Dihydroxyanthracenedione - Studies on the compound dihydroxyanthracenedione, which are proceeding in parallel with a phase I POB clinical trial, have shown that, along with other intercalating agents, this agent produces protein-concealed DNA strand breaks in L1210 mouse leukemia cells. The relationship between these molecular lesions and cytotoxicity remains to be elucidated. However, certain correlations appear to be emerging from the data thus far. m-Amsa, o-Amsa, and ellipticine have relatively little cytotoxicity as a function of the number of

strand breaks induced per unit length of DNA. Adriamycin, actinomycin D, and anthracenedione, have relatively high cytotoxicity per number of strand breaks induced. Therefore, there seem to be two classes of compounds, high-toxicity intercalators and low toxicity intercalators. Anthracenedione appears to be relatively inefficient in producing the strand breaks. In order to double the number of strand breaks per 10^6 nucleotides, one must increase the concentration of anthracenedione by a factor of 100. The reason for this is unclear but may relate to drug penetrance into cells. We are presently investigating other intercalating compounds by the same methods to examine this correlation further.

6. Platinum - The use of atomic absorption spectroscopy to evaluate levels of elemental platinum, mentioned above in relation to clinical problems, has been of more utility in relation to cellular resistance and sensitivity measurements. We have measured the whole cell platinum levels in the L1210 K25 subline, which is sensitive to platinum, and in a platinum resistant derivative, ZCR9, which by all measurements to date is a drug exclusion mutant. The resistant line was developed in vitro and appears to be resistant on the basis of lower intracellular drug concentrations rather than altered DNA damage, repair, or intracellular distribution patterns. The whole cell platinum levels are 2-3 times higher in the sensitive line than they are in the resistant line. This result is compatible with other ratios that have been measured by Drs. Kohn and Zwelling which are in the range of 2 - 2.5. These measurements include the interstrand crosslink frequency and relative cytotoxicity.

Significance to Biomedical Research and the Program of the Institute:

The utility of precise pharmacokinetic measurements of drugs that are being administered to patients, and of ancillary compounds, is obvious with respect to improving the treatment of those patients. The techniques being developed in this laboratory permit such measurements. The explication of resistance to chemotherapeutic agents can prove to be an invaluable tool both for circumventing such resistance and for evaluating diverse biological properties of tumor cells. The basic studies which we have embarked upon, regarding both enzymatic measurements and physico-chemical aspects of drug sensitivity and resistance, should yield principles of fairly broad application in the field of cancer chemotherapy. The development of reliable in vitro measurements of tumor sensitivity to chemotherapeutic agents on an individualized basis would be an invaluable resource in developing chemotherapy programs tailored to a particular patient's tumor. In economic terms, the application of such an in vitro screen to the evaluation of new cancer chemotherapeutic agents would conserve a great many of the resources that are presently consumed in evaluating each agent.

Proposed Course:

It is our intention to pursue molecular mechanisms of sensitivity and resistance in tumor cells using the methods that are outlined above, and to continue to try to elucidate the problems we have observed with regard to individualized in vitro screening for tumor sensitivity. Hopefully, this elucidation will allow an improved testing system. On a clinical level, we plan to continue our efforts in developing new assays where appropriate, to study the pharmacokinetics of anticancer agents in man and in lower animals, and to continue studies relating these measurements to both cytotoxicity and antitumor effect.

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5. Kohn, K.W., Ross, W.E., and Glaubiger, D.: Ellipticine. In Hahn, F.E. (Ed.): Antibiotics V/2. Mechanism of Action of Antieukaryotic and Antiviral Compounds. Heidelberg, Springer-Verlag, 1979, pp. 195-213.
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7. Von Hoff, D.D., and Johnson, G.E.: Initial experience with the human tumor stem cell system: Potential and problems. In Salmon, S. (Ed.): Human Tumor Cloning in Vitro. New York, Alan Liss and Co., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06870-04-PO
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Regulation of Erythroid Differentiation in Normal and Leukemic Hematopoietic Cells		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	A. Deisseroth, Head, Experimental Hematology Section D. Colbert, Cancer Expert D. Hendrick, IPA Investigator* U. Bode, Investigator J. Fontana, Investigator D. Wright, IPA Investigator+ T. Maniatis Y. Kan G. Stamatoyannopoulos D. Weatherall T. Rutherford J. Tischfield E. Schiffman	PO C PO C PO C PO C PO C PO C Cal. Inst. Tech. U.C.S.F. Univ. Wash. Oxford Univ. Oxford Univ. Univ. Georgia LDBA D
COOPERATING UNITS (if any) Cal. Inst. Tech; UCSF; Univ. of Washington; Oxford U.; Univ. of Georgia; Lab. of Developmental Biology and Anomalies, NIDR. *Present Address: 3854 Alturas-Babson Park Cutoff, Lake Wales, Fla. 33853 +Present address: WRAMC, Wash. D.C.		
LAB/BRANCH Pediatric Oncology Branch		
SECTION Experimental Hematology Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 6	PROFESSIONAL: 4	OTHER: 2
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SUMMARY OF WORK (200 words or less - underline keywords) The <u>genetic mechanisms</u> governing <u>hematopoietic differentiation</u> in normal and leukemic erythroid and myeloid cells are being studied through use of cell lines which contain markers of human hematopoietic differentiation. Hybrid mouse erythroleukemia cell lines which contain the chromosome bearing the <u>human alpha globin genes</u> as the only human chromosome have been derived using human donor cells which differ with respect to their epigenotype, stages of development and genetic composition. A series of such hybrid cells, each containing human alpha globin genes from patients in whom non-deletion mutations have led to altered alpha globin gene expression, has been constructed. The various steps of <u>gene expression</u> are being characterized using <u>Southern and northern nucleic acid transfers</u> as well as <u>2 dimensional gel chromatography of non-histone chromosomal proteins</u> . Similar techniques are being applied to HL60, a continuously proliferating human promyelocytic leukemia cell line, in hopes of understanding the <u>regulation of markers of monocyte and of myeloid differentiation</u> in these human leukemia cells, as well as the process which governs commitment to normal <u>maturation in normal cells</u> which is defective in the HL60 cells.		

Objectives:

1. To study the mechanisms regulating the expression of one marker of erythroid differentiation, globin gene expression, as a model for the regulation of expression of markers of hematopoietic differentiation in human normal and leukemic states.
2. To establish continuously proliferating cell lines in which the expression of globin genes from human donor cells of different epigenotypes and genetic compositions can be studied, with respect to the mechanism by which expression of the gene is determined.
3. To correlate functional studies of expression of the human globin genes in hybrid mouse erythroleukemia cells with restriction endonuclease studies (of DNA and RNA from the hybrid cells) in order to identify the precise genetic determinants which control hematopoietic differentiation on a molecular level.
4. To isolate cell lines which can be used to characterize the determinants which govern the transition of expression of embryonic, fetal, and adult globin structural genes which occurs during intrauterine and post-natal life.
5. To relate this detailed analysis of one marker for erythroid differentiation to our understanding of the genetic program which governs differentiation in all hematopoietic cells and the way in which defective expression of this program leads to neoplastic states of hematopoietic cells in man.
6. To establish systems in which the development of complex cellular functions which are characteristic of mature myeloid cells can be studied.
7. To characterize the genetic program which governs myeloid differentiation through the use of hybrid cells derived from cells which are committed or not committed to myeloid differentiation. Specifically, to develop information about the organization of this program: Is it on one or many chromosomes? Are these markers characteristic of myeloid differentiation expressed coordinately? What is the temporal sequence of appearance of each of these markers and how are the complex functions, like chemotaxis, generated in these cells?
8. To determine if the genetic mechanisms which govern expression of cellular functions characteristic of mature hematopoietic cells are similar to those regulating expression of differentiated functions found in non-hematopoietic differentiated cells.
9. To determine if the genetic mechanisms governing differentiation in normal hematopoietic cells can explain the aberrant patterns of differentiation and maturation seen in leukemic states.

Methods Employed:

The techniques of cell biology (cell culture and cloning, chromosomal analysis by light and fluorescent microscopy, velocity sedimentation fractionation of hematopoietic cells at different steps of differentiation, mutagenesis, functional assays of myeloid differentiation, isozymal analysis, radioisotopic analysis of enzyme activity and protein synthesis, cell fusion, and development of in vitro systems for the study of gene expression) are combined with those of molecular biology (molecular hybridization assays for the presence and expression of unique gene sequences, oligo dT chromatography of mRNA, hydroxylapatite chromatography of DNA, synthesis of cDNA corresponding to the globin mRNAs of man and mouse, density gradient ultracentrifugation, gel electrophoretic separation of nucleic acids, restriction endonuclease mapping, Burke-Sharp analysis, R-loop mapping, Southern and northern transfers, cell-free systems for the translation of putative mRNA populations from hybrid cells, 2-dimensional gel electrophoresis of non-histone chromosomal proteins) in order to study mechanisms governing regulation of gene expression in mammalian cells. A tissue culture laboratory equipped with environmental control devices is employed, as well as a complete laboratory for the study of gene expression at the molecular level.

Major Findings:

A. Identification of a Cis-Effective Genetic Mechanism Which Restricts Expression of Human Alpha Globin Genes In Non-Erythroid Cells

We have developed a system which can be used to study the mechanisms which may govern the expression of human α globin genes in human erythroid and non-erythroid hematopoietic cells. Human chromosome 16, which has been shown to bear the human α globin genes, is introduced by cell fusion into mouse erythroleukemia (MEL) cells to generate continuously proliferating cell lines which permanently retain the human α globin genes. We have shown that hybrid diploid MEL cells with human α globin genes from erythroid donor cells express these genes fully through globin chain synthesis while hybrid MEL cells containing a near diploid complement of MEL chromosomes as well as human α globin genes from non-erythroid human hematopoietic donor cells contain very low levels of human α globin mRNA and no detectable human α globin chains. The levels of human α globin mRNA in these hybrid cells were found to depend on factors present in the MEL recipient cell as well as on the differentiated state of the human donor cell. Since the hybrid MEL cells which contain human chromosome 16 from a non-erythroid donor cell in the absence of all other human chromosomes are devoid of human alpha globin chains, and exhibit human alpha globin mRNA levels which are 1/25 of that seen when the human chromosome 16 comes from an erythroid cell, the data suggest that the mechanism which mediates the decreased potential of human α globin genes in non-erythroid human hematopoietic donor cells is cis-effective and that the pattern of

expression of α globin genes in the hybrid diploid MEL cells is dependent on the differentiated state of the human donor cell.

B. Isolation of Hybrid Cell Clones that Contain Deletion and Non-Deletion Defects of Alpha-Thalassemia in Man

We have succeeded in isolating hybrid mouse erythroleukemia cell clones, containing chromosomes from a patient with hemoglobin H disease, which exhibit either deletion or nondeletion mutations of the human alpha-globin genes. Although classical descriptions of this disease had emphasized that three of the four alpha-globin genes are deleted, this patient exhibited the hallmarks of the disease (anemia, microcytosis, hemoglobin H level of 30⁰/o, and an alpha/beta globin chain synthetic ratio of 30⁰/o) even though only two of the four human alpha globin genes had been found to be missing.

Each hybrid cell contained human chromosome 16, which contains the human alpha globin gene in man, as the only human chromosome. Analysis of one of these hybrid clones that had retained a human chromosome 16 from the patient's cells showed that both human alpha-globin genes had been deleted. Several clones of another hybrid cell had retained a human chromosome 16 from the patient's cells, which contained both human alpha-globin genes on an EcoRI fragment of 23 kilobases (Kb). These latter hybrid clones showed the presence of human globin chains at detectable but low levels. These data unequivocally prove that the genetic defect responsible for the decreased human alpha-globin gene expression in the human donor cells does not arise from an interaction of the human alpha-globin genes with a genetic locus located on a human chromosome other than human chromosome 16. Thus, these studies show that there are two different types of human chromosome 16 in this patient and that the nondeletion mutation of human alpha-globin genes leading to hemoglobin H disease in this patient acts in cis to the two alpha-globin genes remaining in his cells.

This work has also established that the low level of expression of the human alpha-globin genes observed to be present in the patient's erythroid cells is also observed after the transfer of these genes from peripheral blood mononuclear cells to the recipient tetraploid mouse erythroleukemia cells. The combination of cell fusion with techniques of restriction endonuclease analysis that we have used in this study may therefore permit correlation of disorders found in the thalassemic patients with the structure and expression of their globin genes seen after introduction into mouse erythroleukemia cells. Such hybrid clones can be used to distinguish between genetic mechanisms acting in cis or in trans to the structural genes, as well as serving as vehicles for generating the chromatin, DNA, RNA, or proteins necessary for the study of such defects at the molecular level. Hopefully, further studies of these and other genetic defects in this hybrid mouse erythroleukemia cell system will permit definition of the exact manner

in which structural changes affecting the expression of the human globin genes manifest themselves as disease states in man.

C. Identification of an Acquired Defect on Human Chromosome 16 Which Leads to Non-Deletion Hemoglobin H Disease in Man

An acquired defect in human alpha globin gene expression (α/β globin mRNA = 0.06, α/β globin chains = 0.08) was found in a patient with a myeloproliferative disorder associated with anemia, a dimorphic population of peripheral blood erythroid cells, hemoglobin H inclusions and a 3.00% level of hemoglobin H. No deletions of human alpha globin genes were present in the marrow cells of the patient. BamHI and EcoRI double digests of the patient's DNA generated fragments containing human alpha globin genes which were 13 (normal size) and 16.5 kilobases in size. Dimethyl sulfoxide induced hybrid tetraploid MEL cells, which contained human chromosome 16 from the peripheral blood cells of this patient with acquired hemoglobin H disease, in the absence of all other human chromosomes, did not have detectable levels of human alpha globin chains. Similar hybrid tetraploid MEL cells with human alpha globin genes from normal donors have detectable levels of human alpha globin chains. The hybrid MEL cells with human alpha globin genes from the patient contained low levels of human alpha globin mRNA which were 1/16th of those found in hybrid MEL cells with human chromosome 16 from normal individuals. These results suggest that the reduced human alpha globin mRNA and human alpha globin chain levels found in this patient and in these hybrid cells is due to a novel defect which is syntenic to the human alpha globin genes, and does not involve deletions of globin coding regions.

D. Study of Non-Histone Chromosomal Proteins in Hybrid MEL Cells Containing Human Chromosome 16

In this laboratory initial attempts to use preparative procedures to isolate and characterize non-histonal proteins by chromatographic techniques met the known difficulties of low separation power and recovery yield. Therefore, an analytical system was developed consisting of electrophoretic separation and isoelectric focusing of proteins in an acrylamide gel between pH 4.5 and 8.0 and then separating the proteins by a discontinuous acrylamide gradient in SDS.

A two dimensional gel electrophoresis system has been developed for the analysis of non-histonal chromosomal proteins found in the hybrid cell with respect to their species origin. The first dimension utilizes isoelectric focusing on slab gels. 150% sodium dodecyl sulfate and polyacrylamide is used in the second dimension further to fractionate these proteins. 450 distinct electrophoretic species have been identified from the parent erythroleukemia cell. When these patterns from the MEL cells are compared to those seen in a hybrid MEL cell which contains human chromosome 16 as the only human chromosome,

electrophoretic species are seen which were not present in the MEL cells. Five out of 6 independently derived hybrid cells which contain human chromosome 16 also contain this non-histone chromosomal protein, which is also seen in 2-dimensional gels of the human parent cells. These results show that the expression of non-histone chromosomal proteins in interspecific hybrid cells exhibit a co-dominant pattern of expression. In addition, this system has permitted us to identify a non-histone chromosomal protein which is associated with the presence of human chromosome 16 in the hybrid cells.

E. The Development of Chemotactic Responsiveness in Myeloid Precursor Cells: Studies With a Human Leukemia Cell Line

The human promyelocytic leukemia cell line, HL60, has been shown to acquire morphologic and functional features of mature neutrophils (PMN) during *in vitro* culture with polar compounds such as dimethyl formamide (DMF). We have used HL60 cells during DMF induced differentiation to study the development of chemotaxis, a complex, integrated function of mature PMN. Uninduced HL60 cells lack chemotactic function, but when incubated in log phase growth (5×10^4 /ml) with DMF (120mM), they develop predictably during 6 day culture into cells capable of directed migration in response to the chemotactic peptide, f-met-leu-phe (fMLP). In acquiring chemotactic function, HL60 develop the functional components of chemotaxis characteristic of normal PMN: surface adhesiveness, spontaneous motility, deformability, membrane receptors for fMLP, and an ability to orient cellular structure in response to chemotactic stimuli. Chemotactic function first appears between 48 and 96 hrs. of DMF induction and is associated not only with the coincidental development of deformability, spontaneous motility, greatly increased fMLP binding, and orientation but also with decreasing cell size and pleomorphism of nuclei. Surface adhesiveness develops earlier (36-48 hrs) and is coincident with a 10-fold increase in protein synthesis, measured by ^3H -leucine uptake, which precedes the full expression of chemotactic function. This early burst of protein synthesis during DMF induced differentiation of HL60 has not been observed in other non-myeloid, DMF inducible cell lines. These studies show that the HL60 cell line can be used to understand the ontogeny of integrated functions characteristic of mature PMN, and this system may provide a useful model for delineating control mechanisms of cell differentiation in the expression of complex cell functions.

Significance to Biomedical Research and to the Program of the Institute:

Our objective has been to clarify the genetic and molecular mechanisms which control the orderly division and maturation of hematopoietic cells. Virtually all leukemic states involve a breakdown in the orderly maturation of one of the types of differentiated cell, or the complete arrest of the ability of these cells to mature normally, resulting in the accumulation of immature committed precursor cells and the cessation of production of the

normal myeloid, erythroid, or lymphoid cells necessary for the survival of the patient. In the hybrid cell system we have used, the pattern of expression of the human alpha globin genes depends on the differentiated state of the donor cell. We can also conclude that there is something associated with the associated with the restriction of expression of human alpha globin genes in non-erythroid hematopoietic cells before transfer, which must be linked to human chromosome 16 and which maintains the restriction after many passages in the MEL cell. Whether these differences in human alpha globin gene expression arise from differences in the DNA itself or from some other type of fixed chromosomal state which acts to stabilize the expression of the human globin genes cannot be concluded at this time. This data does suggest that at least one mechanism limiting expression of human alpha globin genes in non-erythroid hematopoietic cells is cis-effective.

Although our experiments have suggested that restriction of human alpha globin gene expression in non-erythroid human hematopoietic cells arises from a genetic determinant which acts in cis to the human alpha globin genes, our results do not rule out the existence of other mechanisms which are responsible for limiting the expression of these genes in non-erythroid hematopoietic cells. We have not specified the molecular basis for this process, which may affect transcription or change the rates of degradation, processing or transport of transcribed messages. Further experiments are being conducted to determine if the hybrid cloned MEL cells which have human alpha globin genes derived from erythroid or non-erythroid cells also contain differences in the structure of these genes and their flanking sequences, or exhibit differences with respect to the transcription of these genes or the processing of the primary transcripts. Studies are also in progress to determine if the presence or absence of human alpha globin chains in the hybrid MEL cells is related to the relative levels of human alpha globin mRNA found in these hybrid cells or is due to some qualitative difference in these message populations.

The studies using hybrids which contain human alpha globin genes from donors with altered levels of expression of these genes have shown that our hybrid cell system may be useful for establishing the genetic mechanisms underlying these defects. The experiments in which 2-dimensional gel electrophoresis has been applied to the analysis of hybrid cells has shown that we now can search for regulatory proteins which govern the expression of human alpha globin genes.

Previous studies of normal myeloid differentiation have used heterogeneous cell populations because pure populations of precursor cells could not be obtained. With heterogeneous cell populations, it has not been possible to define clearly the role of cellular interactions or to identify those cells which are involved in a specific differentiative event. Kagan et al., studying human bone marrow cells separated into fractions enriched with cells at various stages of myeloid differentiation, demonstrated that chemotactic responsiveness was generally limited to cells in fractions

enriched for mature band and segmented forms. Lichtman and Weed, also using human marrow cells, found that myeloblasts and promyelocytes displayed a high degree of cellular rigidity, low adhesiveness to glass and plastic; and a slow rate of cell spreading and motility. At the myelocyte stage, adhesiveness, motility, and phagocytosis, although not prominent, became evident, and the ability to migrate and phagocytize increased significantly at the metamyelocyte stage. These findings correlate with our findings with the HL60 cells; orientation and migration toward a chemotactic stimulus was found to appear when the induced HL60 cells began to acquire the morphologic features of myelocytes, between day 2 and 4 of induction.

HL60 cell cultures have provided a unique model for the study of myeloid differentiation. The morphologic changes which occur in HL60 cells during induction mimic normal myeloid differentiation, and as shown in our studies, HL60 cells can be induced to acquire those attributes which are necessary for the integrated function of chemotaxis - i.e., adhesiveness, orientation, presence of chemotactic factor receptors and directed motility. As noted above, uninduced HL60 cells were found to bind the chemotactic peptide, f-MLP, to a limited degree. However, there was a rapid increase in peptide binding which began between day 2 and day 4 of induction and which coincided with the appearance of migration responses. Our studies indicated, furthermore, that the ability of cells to bind chemotactic factors is not sufficient for the expression of chemotaxis in the absence of deformability of the cells. While induced HL60 cells have many morphologic and functional similarities to their granule content, we were unable to demonstrate clearly the development of secondary granules in differentiated HL60 cells, nor were we able to detect the specific protein markers for secondary granules in differentiating HL60 cells, lactoferrin or B₁₂ binding protein, either in uninduced or induced HL60 cells. This finding is of interest because it has been proposed that mobilization of secondary granules and fusion of their membranes with the plasma membrane of neutrophils may replenish the supply of chemotactic factor receptors during normal chemotactic responses. Our present studies, however, suggest that the mobilization of secondary granules does not have a permissive role in chemotaxis.

The induction of chemotactic responsiveness in HL60 takes place following a burst in protein synthesis. This is in marked contrast to the mouse erythroleukemia cell in which an increase in total protein synthesis is not demonstrated with induction. It is likely that this burst of protein synthesis in HL60 represents a part of the elaboration of proteins required for motility and for sensitivity to chemotactic stimuli.

The induction of specific membrane receptors as well as surface glycoproteins has been demonstrated in HL60 upon addition of dimethyl sulfoxide. Studies are underway to determine whether the stimulation of protein synthesis observed in our studies is necessary for the induction of the mature granulocyte phenotype and to determine the extent to which proteins synthesized serve structural and contractile functions.

Classification of the genetic mechanisms underlying these control processes in normal hematopoietic differentiation, as well as identification of the defective locus of control in the transformed state, may contribute to our ability to understand and effectively treat these disorders.

Proposed Course:

Future work in this system will be directed to an immediate extension of the studies with HL60 to establish the following points:

1. Is the myeloid program on one or many chromosomes?
2. Is the HL60 cell, once exposed to the inducer DMF, committed to expression of myeloid phenotypes in the absence of the inducer and what is the time course of this event?
3. Is the HL60 cell capable of expression of markers for other lines of hematopoietic differentiation. This point will be studied by the use of a variety of different inducers as well as cell fusion to test the potential for expression of genes coding differentiated markers of erythroid and myeloid differentiation?
4. The use of the techniques of molecular biology (recombinant DNA and Northern transfers) will be used to analyze the events occurring during the commitment and differentiation of the HL60 cells in order to identify the molecular basis for the defective pattern of differentiation underlying this evolution of myelogenous leukemia in man.

We are currently engaged in the analysis of hybrid MEL cells which contain human alpha globin genes derived from donor cells of different developmental stages. We are also using human donor cells in which the human non-alpha (gamma-delta-beta) structural gene complex, which we had shown to be localized on human chromosome 11, has been translocated to other chromosomes bearing genetic markers which can be used for selection of genetically stable MEL hybrid cell lines containing the human non-alpha globin structural genes. Such hybrid MEL may be of use in characterization of the differential expression of the non-alpha and alpha globin structural genes during different stages of development.

The discovery of genetic factors on a chromosome which restrict the expression of globin genes on that chromosome would have obvious relevance to the understanding of the genetic mechanisms responsible for the differential expression of the human non-alpha globin genes. Our previous work provided formal proof that the human gamma and beta globin structural genes are located on the same chromosome (human chromosome 11). During adult life, only a small percentage of the non-alpha globin mRNA and globin chains is composed of human gamma globin mRNA or chains. During fetal life, the non-alpha globin coding sequences present in the cytoplasmic RNA

of erythroid cells are composed primarily of human gamma globin mRNA. The mechanisms responsible for the differential regulation of these two globin structural genes, present very close together on the same chromosome, probably are mediated by events which ultimately have their origin in changes in the genomic DNA in proximity to these two structural genes on chromosome 11. Therefore, we plan to approach the study of the differential expression of the human gamma and beta globin genes by isolation of mouse erythroleukemia hybrid cells into which human chromosome 11 has been permanently introduced. We plan to use human donor cells in which the portions of human chromosome 11 containing the human gamma and beta globin genes have been translocated to human chromosomes 11, 16 or the human X chromosome. Hopefully, such studies will permit us to find ways of eliciting expression of the human globin gene in adult hematopoietic cells of patients afflicted with molecular diseases in which such expression would be of potential clinical benefit.

We plan to use the availability of the hybrid MEL cells which contain human chromosome 16 from erythroid hematopoietic donor cells from normal individuals to characterize and isolate the primary transcript in the mature alpha globin mRNA pathway for the human alpha globin gene. The availability of similar hybrid MEL cells containing human chromosome 16 from non-erythroid hematopoietic donor cells will permit us to determine if the decreased potential for expression of the human alpha globin genes seen in the latter hybrid MEL cells arises from an abnormality in mRNA processing or transcription.

We will also use the MEL hybrid cell which contains the erythroid human alpha globin gene to determine the precise initiation and termination sites within the human alpha globin gene, as well as to attempt to isolate the alpha globin mRNA precursor in order to study the role of RNA-protein interactions and their role in precursor processing and mRNA stability.

We will use the hybrids we now possess as well as others we are now in the process of constructing to study the precise location of genetic lesions within the gene, and the functional cellular level (transcription, processing, or translation) at which the process of alpha globin production is blocked in patients afflicted with various types of non-deletion alpha thalassemia. The same or similar type of question will be attempted in hybrid MEL cells which contain human alpha globin genes and human beta globin genes from normal human donor cells of different stages of development so as to determine at what level and the mechanism by which regulation of the globin genes specific for different stages of development takes place.

These goals will be implemented by the development of specific hybridization probes for various regions (introns, exons, flanking regions, splicing regions, etc.). To find such probes, plasmid subclones of the human alpha globin gene developed by T. Maniatis will be digested with a battery of restriction enzymes, and subjected to agarose gel electrophoresis. Fragments which do not cross react with the mouse alpha

globin genes (genomic clone donated by P. Leder) will be identified by competition experiments as hybridization probes for various regions of the human α globin gene. We will utilize ^{32}P labeled nick transcripts of these regions in hybridization assays to determine the size of the precursors to mature human alpha globin mRNA present in RNA extracted from hybrid MEL cells containing human chromosome 16. By using probes for the two intron regions we will determine which species represent the primary transcript and their order of processing. We will also use the exon and intron probes to determine the steady state nuclear concentration of transcripts from each region. The intron DNA fragments will also be used to construct DNA affinity columns so as to enrich for human alpha globin precursor RNA for further characterization and utilization for in vitro RNA reconstruction experiments (to determine the effect of various protein fractions on RNA, processing, structure, and stability). The enriched precursor fractions will also be used in Burke-Sharp analyses in order to define the precise initiation and termination sites along the gene.

We will screen by Southern hybridization analysis, the DNA from alpha thalassemic patients thought to have non-deletion mutations. Chromosome 16 from such non-deletion blood samples will be transferred to the mouse erythroleukemia cell and the level of globin protein, message, and mRNA precursor determined in order to define the level of the defect. The level of defect will be correlated with modifications within the globin gene in the hope of defining and correlating specific DNA sequences with the regulation of alpha globin production. It is hoped that this work will permit a deeper understanding of the molecular mechanisms which control hematopoietic differentiation in normal and abnormal hematopoietic cells.

Publications:

1. Deisseroth, A., Bode, U., Fontana, J., and Hendrick, D.: Expression of human alpha globin genes in hybrid mouse erythroleukemia cells depends on the differentiated state of the human donor cell. *Nature* 285: 36-38, 1980.
2. Deisseroth, A., Bode, U., Lebo, R., Dozy, A., and Kan, Y.W.: Isolation of hybrid cell clones that contain deletion and non-deletion defects of alpha-thalassemia in man. *Blood* 55: 992-996, 1980.
3. Fontana, J., Wright, D., Schiffman, E., Corcoran, B., and Deisseroth, A.: The development of chemotactic responsiveness in myeloid precursor cells: studies with a human leukemia cell line. *Proc. Natl. Acad. Sci. USA*, in press.

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SUMMARY OF WORK (200 words or less - underline keywords) Experimental approaches to the treatment of <u>meningeal leukemia</u> and other <u>meningeal and non-meningeal CNS neoplasms</u> are explored. A unique <u>subhuman primate model</u> which allows sterile, repetitive access to the <u>cerebrospinal fluid</u> is utilized to study the <u>CNS pharmacokinetics</u> of various <u>intrathecally and intravenously administered chemotherapeutic agents</u> ; to evaluate the <u>neurotoxicities</u> attendant upon various <u>chemotherapeutic and radiotherapeutic treatments</u> ; and to evaluate and screen, in a <u>preclinical setting</u> , newer <u>CNS treatment modalities and drug schedules</u> . Information gained from studies with this model is then applied to the design of clinical protocols used to treat patient with <u>meningeal and non-meningeal CNS malignancies</u> .																																									

Objectives:

1. To explore a subhuman primate model which provides repetitive access to the cerebrospinal fluid and allows detailed study of the pharmacology and neurotoxicity of chemotherapeutic agents used to treat CNS malignancy.
2. To study the CNS pharmacokinetics of currently employed and potentially useful CNS antineoplastic agents.
3. To assess the neurotoxicity of chemotherapeutic agents used in the treatment of CNS malignancy.
4. To better understand the physiology of the blood-brain barrier, using the subhuman primate model.
5. To utilize neurophysiologic and neuropharmacologic information gained in the experimental primate system as a basis for designing new clinical approaches to the treatment of CNS malignancy in man.

Methods Employed and Major Findings:A. Pharmacokinetic Studies Using The Subhuman Primate Model

1. Background – Rational approaches to the treatment of central nervous system malignancies demand precise knowledge of the pharmacokinetic behavior of chemotherapeutic agents in CSF. Meaningful pharmacokinetic investigations require animal systems which approximate the human situation. We developed a sub-human primate system which avoids the above shortcomings. Our model system allows for repetitive sterile sampling of CSF over extended periods of time in unanesthetized animals. Studies to date have demonstrated that this model provides CNS pharmacokinetic data which are similar to that obtained in man. The model involves the subcutaneous implantation of an Ommaya reservoir in rhesus monkeys. The reservoir chamber is placed over the occiput and is attached to a silastic Pudenz catheter. The latter passes through the foramen of Magendie connecting the reservoir with the fourth ventricle. This system allows drugs injected into the reservoir to be distributed throughout the ventricular CSF, a process facilitated by barbotage (i.e., of the reservoir chamber). Scintillation photographs of technitium ^{99m}-labelled human serum albumin injected into the reservoir have documented that pumping the reservoir insures complete mixing of the fluid in the reservoir with that within the entire ventricular system. Sampling of ventricular fluid from the reservoir is therefore accomplished by pumping the reservoir chamber prior to removal of CSF.

2. Studies on the CSF Pharmacokinetics of Methotrexate - The applicability of this primate model to the study of CNS pharmacokinetics in man was confirmed in studies which compared the CSF pharmacokinetics of methotrexate in the monkey with that in man. In these studies, methotrexate concentrations were measured utilizing the dihydrofolate reductase inhibition assay. We first examined the disappearance of methotrexate from CSF in plasma following intraventricular injection in the monkey. The methotrexate disappearance curves obtained in the monkeys were strikingly similar to those obtained in humans given comparable intrathecal doses. In both species, the clearance of the drug follows a tri-phasic pattern. The first (alpha) phase is a distribution phase which occurs in the first hour after injection. The second (beta) phase occurs between 4 and 38 hours, and the third phase occurs between 48 and 96 hours post injection. Both the qualitative and quantitative (for example, CSF:plasma ratio) aspects of the disappearance curve are similar in both species.
3. Experimental Methods of Improving CNS Antifol Therapy - After confirming the validity of the subhuman primate model we have investigated potential methods of improving methotrexate therapy to the central nervous system. Several approaches have been studied, the most recent of which are discussed in detail below.
 - a. Improved Cerebrospinal Fluid Drug Distribution Utilizing Hyperbaric Intrathecal Methotrexate - Hyperbaric chemotherapy utilizes the effect of gravity on an injected bolus of drugs which is heavier than the fluid into which it is injected. For lumbar injections, the drug is reconstituted or dissolved in a heavier than cerebrospinal fluid vehicle and the patient is placed in Trendelenburg position for one or two hours. The hyperbaric technique previously had been shown to improve intrathecal administration of amphotericin and radionuclides which were attached to high molecular weight compounds (^{99m}Tc -albumin, ^{131}I -albumin, etc). We have used the subhuman primate model to evaluate the hyperbaric method of intralumbar drug administration of methotrexate. In our studies, ventricular cerebrospinal fluid methotrexate levels were determined on CSF samples from four animals, each of whom had been given methotrexate twice, once with Elliot's B solution (artificial CSF) as the diluent and once with 10% dextrose in water as the diluent (a heavier-than-CSF fluid). Following each intralumbar injection, the animals were placed in the Trendelenburg position. The results of these studies demonstrated that higher antifolate concentrations are achieved in ventricular cerebrospinal fluid when methotrexate is given by the hyperbaric method. These findings suggest that use of the hyperbaric technique may have clinical advantages.

- b. Studies on the Intravenous High-Dose Methotrexate Approach to CNS Therapy - We have utilized the subhuman primate model to evaluate the feasibility of utilizing high-dose intravenous methotrexate infusions to treat CNS leukemia. Our goal is to replace intrathecal methotrexate as it is currently administered clinically with effective systemic therapy, since the latter route has theoretical advantages. First, it is easier to accomplish and less traumatic than intrathecal therapy. Second, this route is more physiologic, in that the drug is carried to the tumor by the same vascular channels that support its growth. Third, the use of prolonged infusions permits a "concentration x time" ($C \times T$) approach, i.e., the provision of therapeutic concentrations of drug within the CSF for a prolonged period of time. We have infused subhuman primates with doses of high-dose methotrexate comparable to those currently being utilized in our patient trials. Our studies in the subhuman primate have confirmed the feasibility of the intravenous high-dose methotrexate approach to CNS therapy. We have been able to show that: 1) Therapeutic levels of methotrexate can be achieved in the cerebrospinal fluid by the intravenous approach without significant systemic toxicity; 2) the mean CSF to plasma drug ratio from ventricular and lumbar sampling sites is similar, suggesting that systemic chemotherapy has the advantage of providing consistent drug concentrations throughout the CSF axis; 3) prolonged intravenous methotrexate infusions provide a $C \times T$ approach; and 4) there is a linear relationship between plasma methotrexate concentrations and CSF drug levels achieved. The information gained from our studies on intravenous high-dose methotrexate in subhuman primates has formed the basis for our current meningeal leukemia clinical trials (both in the overt and prophylactic settings).
- c. CSF Pharmacokinetics of Aminopterin - In addition to studies with methotrexate, the primate model has been used to investigate the CSF pharmacokinetics of other antifolates. Recent experiments have focused on the CNS pharmacology of aminopterin. Aminopterin is more potent than methotrexate in that an extracellular concentration of 1/5th to 1/10th that of methotrexate achieves the same anti-tumor effect. In contrast, the concentrations causing acute neurotoxicity in dogs appear to be the same for methotrexate and aminopterin. It is possible, then, that aminopterin given at 1/10th of the methotrexate dose may be equally effective against CNS leukemia and considerably less toxic, provided that the disappearance kinetics of the two drugs are similar. To test this hypothesis, we administered aminopterin intraventricularly in the primate model at 1/5th the dose of methotrexate. Interestingly, the disappearance from CSF was significantly

slower for aminopterin than for methotrexate, such that the concentration curves have crossed each other approximately 24 hours after injection, resulting in several fold higher levels of aminopterin between 48 and 96 hours after injection. These observations suggest that when compared to methotrexate, intrathecal aminopterin may provide more sustained antifolate levels in the CNS at 1/5th the dose and with potentially less neurotoxicity. If the results achieved in this model are predictive for man, aminopterin could conceivably prove to be a safer intrathecal chemotherapeutic agent, and possibly one which requires fewer lumbar punctures for repetitive administration. Our observations on the CSF pharmacokinetics of aminopterin in the subhuman primate have provided the stimulus for an ongoing phase II trial of this agent in man.

4. Studies of CSF Pharmacokinetics of Other Antineoplastic Agents

- a. AZQ - AZQ (NSC 182986) is an aziridinyI-benzoquinone, a class of agents showing significant anti-tumor activity in murine tumor systems. AZQ was synthesized in an effort to produce compounds which had sufficient lipid solubility and low ionization properties to allow central nervous system penetration, yet possessed a degree of aqueous solubility adequate for drug formulation and administration. AZQ is of considerable interest since intraperitoneal administration produced significant increases in life span and some cures in the ependymbioblastoma, intracranial L1210 and intracranial P388 model systems. Because of this and other animal data suggesting that AZQ possesses a broad spectrum of activity, this agent is entering phase I testing with the eventual hope that it will prove to be clinically effective against brain tumors. We measured the concentration of AZQ in monkey cerebrospinal fluid to confirm the compound's ability to enter the brain. AZQ, labeled with ^{14}C , was administered by IV bolus to 4 monkeys. The plasma decline of the drug was triphasic; the half lives, determined by sequential stripping of the plasma curve, were 0.5 ± 0.2 hours, $1.8 \pm .45$ hours and 90 ± 46 hours. In the CSF, total radioactivity rose to a maximum one hour after AZQ administration and the subsequent decline roughly paralleled plasma values. The peak CSF radioactivity was 300% of the corresponding plasma value. To determine the amount of AZQ present in CSF as parent compound, chloroform extraction and subsequent thin layer chromatography was performed. Over the period of at least the first 2 hours, greater than 80% of the radioactivity present in the CSF was that of the parent compound of AZQ. These results show that AZQ enters the CSF and achieves concentrations that are substantial compared to plasma levels. These data further emphasize the need for clinical study of

this agent. Hopefully, the high CSF concentrations will be reflected in clinical activity against both brain and meningeal malignancy.

- b. Alteration of Cerebrospinal Fluid Pharmacokinetics of Cytosine Arabinoside (ARA-C) by Tetrahydrouridine (THU) - THU has been shown to increase plasma levels of ARA-C by blocking its enzymatic deamination. We explored the effect of THU on the pharmacokinetics of intrathecally administered ARA-C following either intrathecal (IT) or intravenous (IV) THU administration. These experiments were performed in our sub-human primate model. We first studied the influence of (IV) THU on the entry of ARA-C into the CSF. Following an IV bolus of ^3H -ARA-C (8 mg/kg), negligible quantities of intact drug were detected in the CSF. However, when the same dose of ^3H -ARA-C was administered 30 minutes following an IV bolus of THU (100 mg/kg), intact ARA-C was detected in the CSF, rising to a maximum concentration of $2\ \mu\text{M}$ at 60 minutes. CSF levels of ARA-C then declined with a single half life of 2.5 hours. The effect of THU on the disappearance of intrathecal (IT) ARA-C was also evaluated. When THU (6 mg/kg) was injected simultaneously with ARA-C (1 mg/kg) by the IT route, the concentration of ARA-C in the CSF was increased substantially and its disappearance from the CSF was markedly retarded. Six hours after administration of ARA-C (IT) + THU (IV) or ARA-C (IT) + THU (IT), CSF ARA-C concentrations were 12-fold and 20-fold greater, respectively, than when ARA-C was given alone. This increase appears to be primarily due to inhibition of ARA-C deamination, since a marked decrease in CSF uracil arabinoside (ARA-U) concentration was observed in the presence of THU (either IT or IV). This suggests that the ability of THU to alter CSF ARA-C pharmacokinetics may have potential therapeutic value in man. Use of the combination of THU and ARA-C should be considered for the treatment of meningeal disease in man.
- c. L-Asparaginase (ASNase) Pharmacokinetics and L-Asparagine (ASN) in the Cerebrospinal Fluid (CSF) - ASNase exerts its antitumor activity by hydrolysis of ASN. Both intravenous (IV) and intrathecal (IT) ASNase have been reported to clear lymphoblasts from the CSF of patients with acute lymphoblastic leukemia. To determine the optimal route of administration for the treatment of CNS leukemia, we measured the levels of ASNase and ASN in the CSF of rhesus monkeys after both intravenous and intrathecal doses of ASNase. Following intrathecal administration via the Ommaya reservoir of either E. Coli or Erwinia ASNase, enzymatic activity declined rapidly. The terminal half life of each enzyme was shorter than the measured half life of ^{111}In -labelled diethylene triamine penta-acetic

acid (a marker of CSF bulk flow), which was 6-7 hours. Administration of intrathecal ASNase (1000 IU/m^2) resulted in a complete depletion of CSF ASN for at least 5 days. Although a negligible amount of enzyme activity could be detected in the CSF following IV administration of $6000 \text{ IU/m}^2/\text{day}$ for 3 days of E.Coli ASNase, ASN was completely depleted in the CSF for at least 5 days. Thus both the IT and IV routes of ASNase administration can produce prolonged depletion of CSF ASN. Similar studies were performed in man measuring CSF ASN levels during ASNase treatment. Preliminary results indicate that a similar depletion of CSF ASN occurs in patients undergoing treatment with systemic ASNase. The length of CSF ASN depletion appears to be influenced by the schedule of drug administration. These findings may have implications for the planning of treatment and/or assessment of neurotoxicity in patients with ALL.

B. Studies on the Neurotoxicity of Methotrexate Plus Cranial Radiation:

Observations in the Primate Model - We have been developing a model for methotrexate leukoencephalopathy. In a pilot study, monkeys were given 3500 rads of cranial irradiation, and a continuous CSF methotrexate level greater than 10^{-8} molar was maintained in these animals by biweekly intraventricular injections. Within 4 months, the animals became profoundly neurotoxic and expired; at autopsy, their brains revealed lesions identical to those seen in patients who have developed diffuse necrotizing leukoencephalopathy following cranial irradiation and either intrathecal or intravenous methotrexate therapy. In a recent set of experiments, primates with indwelling Ommaya reservoirs were divided into 2 treatment groups. The first received hemi-brain irradiation (1500 rads) as a single dose; the second received this radiation dose plus biweekly intraventricular methotrexate. Preliminary results have demonstrated the presence of pathologic changes typical of leukoencephalopathy in the irradiated halves of the brains of the animals given methotrexate, with no pathology seen in the unirradiated brain. The animals given radiation alone had no evidence of pathologic changes. These data confirm the synergistic role of methotrexate plus cranial irradiation in the pathogenesis of this entity.

Significance to Biomedical Research and the Program of the Institute:

Rational treatment of central nervous system neoplasms requires knowledge of the physiology of the blood-brain barrier and a clear understanding of the CNS pharmacokinetics of antineoplastic agents. Detailed pharmacological investigation in humans is limited by the lack of a ready route of access to cerebrospinal fluid. The development of the subhuman primate model facilitates such studies in a setting that approximates the

human situation. In addition, the model provides for study of chemotherapy and radiotherapy-related neurotoxicity, allowing for delineation of factors predisposing to toxicity as well as for identification of methods useful in monitoring the development of toxicity. Data obtained in this model has led to a variety of new treatment approaches now being assessed in man. Information gained in the primate regarding the levels of methotrexate achievable in the CSF following intravenous administration has led to current clinical trials. Data suggesting the potential advantages of intrathecal aminopterin over methotrexate have resulted in our plan to study this agent in patients. Our finding that new compounds such as AZQ cross the blood brain barrier suggests that this agent should be studied clinically in patients with brain tumors. In addition, information gained regarding the depletion of CSF asparagine following systemic asparaginase treatment may be of value in the planning of therapy with this agent. Finally, studies of the combination of cytosine arabinoside and tetrahydrouridine suggest that it may be of value in treating meningeal leukemia.

Proposed Course:

Use of the primate model to screen agents of potential value in treating CNS malignancy will continue. Studies of a variety of agents, including the antifols, platinum compounds, nitrosoureas, radiosensitizers, and phase I agents (e.g., 2'-Deoxycoformycin) are in progress. Particular emphasis will be placed on the intravenous approach to the treatment of CNS malignancy, and studies will be designed to assess the penetration of intravenously administered compounds, e.g., methotrexate, into brain tissue. Exploration of various combination chemotherapeutic approaches to treating the CNS will also be continued. Finally, a comprehensive study of post-therapy leukoencephalopathy is underway in an attempt to learn more about those factors which predispose to the development of this syndrome.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM-06890-01-P0
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Lymphoma Biology and Epstein-Barr Virus		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	I. Magrath P. Pizzo G. Tosato R. Spiegel E. Douglass J. Whang-Peng M. Blaese M. Santaella E. Jaffe M. Frank	Senior Investigator Senior Investigator Expert Clinical Associate Clinical Associate Section Head Section Head Investigator Senior Investigator Clinical Director, Head of CIB PO C PO C MET C PO C M C M C MET C LCI I LP C LCI I
COOPERATING UNITS (if any) Metabolism Branch, Laboratory of Pathology (NCI); Laboratory of Clinical Investigation, NIAID.		
LAB/BRANCH Pediatric Oncology Branch		
SECTION -----		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5	PROFESSIONAL: 3	OTHER: 2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINOR ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The primary objectives are to study aspects of the <u>cell biology of lymphomas</u>, particularly as this relates to their normal counterparts in the <u>differentiation sequences of lymphocytes</u>. To this end we attempt to derive <u>cell lines</u> from the tumor cells of <u>lymphoma patients</u> and study these lines <u>in vitro</u>. We are particularly interested in attempting to induce <u>differentiation</u> and in the <u>control mechanisms</u> governing this. We also study the physical properties of <u>membrane lipids</u>, and <u>cytogenetics</u> of lymphomas and derived cell lines. <u>Epstein-Barr virus (EBV)</u> is a major interest, particularly from the aspect of its <u>potential human oncogenicity</u>. The differences between tumor cell lines which contain or lack EBV are being studied, and we hope to determine the types of immature lymphoid cells which may harbor EBV. We are interested in comparing the biochemical and biological properties of different <u>EBV strains</u> and in assessing the possible importance of strain differences to <u>EBV-associated diseases</u>. As part of this work, we have been exploring the ability of EBV to <u>activate mature B cells</u> and induce <u>immunoglobulin secretion</u>. The cellular regulation of this phenomenon and the <u>manifest differences between virus strains</u> in this system are a particular focus.		

Objectives:

1. To obtain tumor-derived cell lines from lymphoma patients and to use these in the study of lymphoma biology.
2. To induce differentiation in vitro and to determine the correlation between cell surface differences and in vitro behavior, including infectibility with EBV and response to plant lectins.
3. To study the cytogenetics of lymphomas and derived cell lines.
4. To examine the thesis that malignant cells have abnormal membrane microviscosity.
5. To compare isolates of the Epstein-Barr Virus (EBV) which have been obtained from different clinical settings (e.g., African Burkitt's lymphoma, American Burkitt's lymphoma, infectious mononucleosis), and to determine whether differences can be discerned which might clarify the diverse biologic and pathologic expressions associated with this virus.
6. To investigate the role of other cofactors (e.g., C-type viruses) in the oncogenic expression of EBV.
7. Since the ability of a virus to remain latent may play a role in oncogenesis, factors which modify virus expression, transcription and production will be investigated.
8. To investigate the immunostimulatory effects of EBV on human lymphocytes: The classes of immunoglobulins secreted in response to EBV stimulation; the role of T-cells in EBV stimulation (both as helpers and independent responders) and whether the response differs with T-cells obtained from seropositive vs. seronegative individuals. In addition, the manner in which different strains of EBV (i.e., transforming vs. infecting) stimulate immunoglobulin production will also be investigated.

Methods Employed:

Cell culture. Primary (i.e., tumor-derived) and established lymphoid cell lines are grown and maintained in RPMI 1640 plus 10-20% fetal calf serum at 37°C. Physical characteristics and karyotype are routinely examined. Immunological techniques are utilized in the study of cell lines including examination for surface immunoglobulin and various surface receptors. Various sub-types of complement receptors are examined (C3b, C4b, C3d) as well as EBV receptors, utilizing purified reagents and rosetting techniques. Cell membrane 'microviscosity' is studied by examining the rotation of a hydrophobic, fluorescent probe, diphenyl hexatriene (DPH), incorporated into cells or cell membranes. Rotation is

measured in terms of fluorescence polarization anisotropy after excitation of probe molecules in a single plane of polarization. Virus producing cell lines are grown in 6-12 liter quantities at different temperatures (32-37°C) for 5-14 days during which time the virus is labelled with ³H-thymidine. Supernatants are harvested, clarified, concentrated by ultrafiltration and ultracentrifugation, and virus purified by dextran velocity and isopycnic sucrose sedimentation. Radiolabelled virus containing bands are ultracentrifuged and dialysed. Purified viruses or cell suspensions are detergent lysed and subjected to enzymatic digestion of protein and RNA followed by phenol extraction and ethanol precipitation. Certain viral DNA preparations are purified by isolation of 55s DNA on sucrose gradients followed by organic extraction. Viral DNA preparations are banded to equilibrium in cesium chloride and have an A260/280 nm ratio of 1.80-1.95. Viral antigens, particles, and biologic activity are assayed by immunofluorescence (EA, VCA, EBNA). Virus particles are quantitated in cells by electron microscopy. EBV infectivity is assayed by induction of EA in RAJI cells while transformation is determined by the genesis of EBNA-containing cord blood lymphoid cell lines following EBV infection. Sheared radio-labelled purified viral DNA is mixed with an excess of sheared unlabelled cellular (or viral) DNA, denatured, incubated at 65°C, and timed-samples assayed by hydroxyapatite chromatography to determine the degree of hybridization. Thermal elution profiles are determined on certain incubation mixtures utilizing graded temperature increments and hydroxyapatite chromatography. Radiolabelled-EBV is digested with purified restriction enzymes, analyzed by electrophoresis on agarose slab gels, and detected by fluorography or autoradiography. Immunoglobulin secretion is studied using the reverse hemolytic plaque assay in which Staphylococcal A protein is coupled to sheep red blood cells (SRBC) and then mixed with washed target cells, incubated on agar, and assayed with rabbit IgG antibody to human immunoglobulin(s) in the presence of complement. Immunoglobulin secreting cells are detected by the presence of lysis of the SRBC - visible as a clear plaque. EBV is preincubated with purified populations of B cells, T cells, and cell mixtures. Proliferative capacity is measured by ³H-thymidine incorporation.

Major Findings:

A. Derivation of Cell Lines

In the last three years we have been able to derive cell lines with a success rate of about 50% at initial presentation and 80% at relapse. In all, 28 cell lines have been derived from 23 American patients with Burkitt's lymphoma, or undifferentiated non-Burkitt's lymphoma. Three cell lines have been derived from West African (Ghanaian) patients with Burkitt's lymphoma. Fourteen cell lines have been derived from the peripheral lymphocytes of normal individuals. These cell lines can be divided into groups according to their EBNA (EBV nuclear antigen) status and karyotype. Fourteen lines are EBNA

negative and 8 EBNA positive, all of which possess specific chromosome abnormalities (8:14 translocation or 14q+). Only in one case was overgrowth of malignant cells with diploid, EBNA-positive cells demonstrated. All lines of normal origin were EBNA positive and diploid. The EBNA positive lines of tumor origin were all derived from EBNA negative tumors. The explanation for this appears to be that a small fraction of the tumor cells undergo differentiation *in vivo* or *in vitro* to give rise to cells which can be infected by EBV. We have shown that EBNA positive cells whether of tumor cell origin (8:14 translocation or 14q+) or of normal lymphocyte origin (diploid) differ from EBNA negative cells. EBNA negative cells have a higher saturation density in RPMI 1640 containing 20% FBS, have smaller cell volume (Coulter channelyzer or narrow angle light scatter), stain more brightly with anti-Ig fluorescinated reagents, and express lower levels of FcIgG, complement (C3) and EBV receptors than EBNA positive cells. In fact, we were able to demonstrate a highly significant correlation between EBV receptors and complement receptors, especially those for the C3b component.

1. Induction of C3 and EBV Receptors - If the small number of C3 and EBV receptor positive cells in American Burkitt's lymphoma cell lines are present as a result of more advanced differentiation in a fraction of the cells, such a differentiation step may be demonstrable *in vitro*. Accordingly, we have studied the influence of agents known to influence lymphocyte differentiation and activation on our EBNA negative cell lines. In two cell lines (EW36 and CA46) we have been able to demonstrate induction of both C3 and EBV receptors by means of methylated xanthines. We have not been able to induce receptors with inducers of cyclic nucleotides or with cyclic nucleotide analogues. However, these compounds may have a role in maintaining levels of receptors once these have been induced, since papaverine (a phosphodiesterase inhibitor) can maintain receptors after removal of theophylline. The mechanism of action of theophylline appears to be two-fold: 1) It induces specific messenger RNA production (actinomycin D specifically inhibits the inducing effect of theophylline); 2) It maintains receptor levels, possibly by influencing the cyclic nucleotide system. The inducing, but not maintaining effect of theophylline can be inhibited by purine, adenine and adenosine. In addition, these compounds can substitute for theophylline in the maintenance of receptors. Further work needs to be done on the importance of the adenosine/adenosine deaminase system in B lymphocyte differentiation, but our data support the possibility that the action of methylated xanthines is not an *in vitro* artifact, but may be working via its influence on purine metabolic pathways and/or purine receptors - consistent with the evidence from other workers that purines and their derivatives have an important role in cell differentiation.

2. The Induced Complement Receptor Differs from that of Normal Lymphocytes - Using "intermediate" reagents prepared stepwise with purified human complement components, we have been able to show that the induced complement receptor on EW36 and CA46 cells differs from that of normal lymphocytes, polymorphs, human red cells and the cells of a patient with nodular lymphoma in two characteristics: 1) The affinity for C3b is several hundred fold less; 2) we cannot demonstrate C4b binding. The possibility that the same receptor is differently oriented or spatially located in mature cells compared to EW36 or CA46 cells cannot be excluded at present, but we can exclude a difference in membrane lipids as an explanation for different orientation, since fluorescence polarization does not differ in cells having C3 receptors from those which lack C3 receptors. These data support the previous conclusions that lymphoma cells which bear complement receptors and EBV receptors (rendering them infectible by EBV) are slightly more differentiated cells than those which do not bear EBV and complement receptors. These cell types are probably the respective cells of origin of African and American Burkitt's lymphoma.

B. The 8:14 Translocation [t(8:14)]

We have been able to identify a t(8:14) in direct tumor material in 10 of 18 patients (55⁰/o) studied. The cell lines of 7 of the 9 patients from whom cell lines had been obtained contained a t(8:14) -- 77⁰/o. The total number of patients with the t(8:14) present in tumor material and/or cell lines was 12 (66⁰/o). Two patients had a 14q+ without an identifiable 8q- in tumor material and 2 patients had a 14 q+ without an identifiable 8q- in their tissue culture lines. A total of 15 patients (83⁰/o) had either a t(8:14) or a 14q+ present in tumor material and/or cell lines. Three of these patients (17⁰/o) had no 14q+ in direct tumor preparations, but the abnormality subsequently was found in cell lines established from these tumors. All of the cell lines from the 9 patients contained either a t(8:14) or a 14q+.

C. Fluorescence Polarization

Whole cell fluorescence polarization measurements of peripheral lymphocytes from normal donors fell within a narrow range, from .268 to .298, with a mean of $.286 \pm .008$. Normal donors tested on repeated occasions showed variation of $\pm .010$. The P-values of neoplastic cells obtained from patients with various types of leukemia and lymphoma were significantly different, and each disease had a specific range of fluorescence polarization values. Mean values were: CLL 0.267, ALL (null) 0.245, ALL (T-cell) 0.247, lymphomas (B-cell) 0.210, lymphomas (T-cell) 0.188. Comparison of P-value of whole cells and isolated membranes of freshly obtained blasts from acute leukemia revealed that in spite of the low whole cell P-values, isolated membrane fractions had similar P-values to normal lymphocytes. The P-values of normal

lymphocytes, however, did not significantly differ regardless of whether the measurement was made on whole cells or isolated membranes. In fact, membrane preparations from cells as diverse as normal circulating lymphocytes, leukemic blasts obtained directly from patients, and a variety of lymphoid cells in long-term culture, regardless of karyotype or presence of EBV DNA, had remarkably similar P-values. Thus, the lower fluorescence polarization of neoplastic lymphoid cells does not appear to be due to a difference in the lipids of the cell membrane.

Since intracellular lipids with low fluorescence polarization anisotropy could account for the low whole cell P-values, we investigated this possibility. As expected from previous reports, histologic and cytologic preparations of fresh tumor material and of cells in culture revealed the presence of vacuoles containing neutral lipids. With some cell homogenates subjected to sucrose gradient separation after nitrogen cavitation, a lipid layer, floating on top of the buffer, was observed. Analysis of this layer showed it to be pure triglyceride with a very low P-value (0.183-0.201), consistent with the proposal that intracytoplasmic lipid accounts for the low P-value of lymphoid tumor cells.

D. Studies with Epstein-Barr virus

1. EBV Strains - We have isolated, purified, and initially characterized an EBV strain from a patient with American BL. We believe that this represents the first EBV isolate (JL-P) from an American BL which has been biochemically analyzed. Hybridization studies using radiolabeled JL-P EBV DNA with AG876, B95-8 and HRL DNAs failed to show any significant difference between the JL-P DNA and that of AG876. A similar association is demonstrated by restriction endonuclease mapping, further suggesting the similarity of the American BL isolate to that which we recently isolated from an African patient with BL (AG876). Since EBV is only associated with a small number of American BL patients (in contrast to virtually all African cases), the similarity of these viruses is intriguing.
2. Cofactors and EBV - The fact that EBV is associated with several human diseases, and that even in Africa less than one child in a thousand who is exposed to EBV at an early age eventually develops Burkitt's lymphoma, suggests that other factors operate in addition to (or instead of) EBV to produce malignant transformation. Among the possibilities are other viruses, specifically the type C oncornoviruses. Type C particles and 70s particles containing RNA-dependent DNA polymerase have been observed in fresh tumor cell lines derived from BL. To examine further the association of oncornovirus with human lymphomas and their derivative cell lines, we have studied 36 African and American lymphomas and/or their cell lines for the presence of EBV and/or oncornovirus proviral

sequences. Three virus probes were used: EBV, amphotropic 1504-A MuLV, and simian sarcoma virus (SiSV-1). DNA from each of the 6 African tumors or cell lines hybridized to 78-94% with radiolabelled-EBV DNA. Of the 14 American Burkitt's tumors, only 2 (14%/0) contained EBV sequences. However, both the amphotropic and SiSV-1 cDNA probes failed to hybridize with any of the tumor or cell-line DNAs. Thus, the contribution of oncornoviruses to the transformation state is not substantiated by this study.

3. Induction of EBV - We have continued our studies on the effect of corticosteroids on the induction of EBV. Previous studies have shown an approximately 10-fold increase in the yield of EBV when P3HR1 cells are grown in the presence of dexamethasone at 32°C. We have further quantitated the effects of steroids and temperature as independent variables in this process by measuring the change in EBV-genome equivalents by reassociation kinetics. Furthermore, similar effects of steroids and/or temperature cannot be demonstrated in other EBV-producing cell lines (B95-8, AG876, JL-P), suggesting that this induction for HR1 is unique. We are also assessing the effect of steroids on the episomal vs. integrated EBV sequences in the HR1 cell line as well as studying several agents which may impair this induction process (Ara-A, V5531). The effect of these agents on RNA transcription is also being investigated.
4. EBV as a polyclonal activator - Initial studies using the B95-8 strain of EBV confirmed the Ig stimulating effect of EBV. In addition it was shown that IgG, IgM and IgA are all stimulated by EBV, and that DNA synthesis is increased in both unseparated lymphocytes and purified B cell populations. Immunoglobulin secretion began on day 3 or 5 and subsequently increased. U-V irradiation of virus completely abrogated this effect. The polyclonal activating effect of EBV is independent of T cells since highly purified B cell populations (containing less than 10% T cells) produced similar numbers of plaques as unseparated populations. Furthermore, admixture of autologous T cells to purified B cells did not alter immunoglobulin secretion, whether or not the T cells were irradiated. This result is of interest since activation of lymphocytes by PWM required the presence of T cells (i.e., helper T-cells).

E. Studies on lymphocytes from patients with infectious mononucleosis

Unseparated lymphocytes from patients with infectious mononucleosis failed to respond with immunoglobulin secretion to either PWM or EBV. However, separated B cell populations had a normal response to EBV (1277X 1.17 Ig secreting cells in IM patients compared to 1631X 1.16 in normals) as did B cells co-cultivated with normal allogeneic helper T cells and PWM. However, admixture of T cells from patients with infectious mononucleosis abolished immunoglobulin secretion by

both autologous and allogeneic B cells incubated with EBV. This effect of IM T cells could be abolished by irradiating them, and we interpret these results as indicating a powerful suppressor effect of IM T lymphocytes.

Further studies on patients with IM indicated that early in the disease (i.e., in the first week) there are increased numbers of circulating cells capable of secreting immunoglobulin (i.e., consistent with the observed hypergammaglobulinemia), whereas during the second week there is a striking reduction in these cells, in both cases compared to normal donors. This strongly suggests that initial B cell activation is brought about *in vivo* by EBV infection, and that this activation is subsequently controlled by the generation of powerful suppressor T cells.

F. Importance of virus strain in B cell activation

Preliminary results have indicated that whereas both transforming (B95-8 and AG876) strains and non-transforming strains (P3HR1) of EBV will stimulate tritiated thymidine uptake in T cells, only the transforming strains induce thymidine uptake in B cells. Nonetheless, both transforming and non-transforming strains will induce Ig secretion in lymphocyte populations, although the non-transforming strains require the presence of helper T cells whereas transforming strains do not.

Significance to Biomedical Research and the Program of the Institute:

Attempts to comprehend lymphomas are doomed to failure if a purely morphological approach is used. As data accumulates, the resemblance of neoplastic lymphoid cells to their normal counterparts becomes more and more striking. Moreover, it is becoming clear that the behavior of lymphomas which have been identified precisely, e.g., by detailed immunochemical phenotypic characterization, can be predicted on the basis of what is known of the normal cell counterpart. Further, response to therapy and the approach to therapy also differs according to cell type when accurately determined, as opposed to utilizing a purely histological approach. Thus, our objective - to comprehend at a cellular level the biology of lymphomas - should result ultimately in an improved ability to separate different pathological entities, therefore to improve our ability to analyze the results of therapeutic trials and, hopefully, to indicate possible novel approaches to treatment.

EBV is closely associated with at least three human diseases - infectious mononucleosis, African Burkitt's lymphoma and nasopharyngeal carcinoma. As such it represents a potential human tumor virus, and clarification of its role in the diseases with which it is associated is of paramount importance. Studies on the induction of EBV with corticosteroids may clarify the relationship of EBV to the host cell since they suggest that the viral genome may be influenced by mechanisms which regulate the

transcription of unique genes in differentiated cells. Such information is pertinent to an understanding of the association between latent virus infection and oncogenesis - both of which are important characteristics of the herpes virus group as a whole. Studies of different strains of EBV will help to determine whether or not differences in virus properties are relevant to the association of EBV with sub-clinical, benign and malignant diseases. Studies of the modification of normal lymphocyte behavior induced by EBV, and T-cell regulation of these phenomena, will provide insights into the spectrum of EBV associated diseases, and may also lead to the definition of specific genes related to different biological properties, e.g., transformation versus immunoglobulin production.

Proposed Course:

The studies in lymphoma biology and EBV will be continued. In particular, we would like to attempt to characterize B-cell malignancies in terms of lymphocyte differentiation antigens. This would involve the generation of monoclonal antisera with mouse myeloma hybridomas. Such reagents would potentially provide objective identification of individual lymphoid malignancies, and lead to definitive answers to such questions as the relationship between undifferentiated B-cell lymphomas of various types. Such antisera may also be useful in studying EBV and its influence on B cells, e.g., are there specific B-cell populations which EBV infects? What are the differences in transcription (in terms of immunologically identified proteins) between lymphocytes which produce Ig in response to EBV but are not transformed, and transformed cells? Further studies are planned in conjunction with the Laboratory of Clinical Investigation on the relationship between EBV and complement receptors, and the significance of this to an understanding of the nature and evolution of virus receptors - a concept of considerable importance in the case of latent viruses which can function as horizontal transmitters of genetic information.

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ANNUAL REPORT OF THE RADIATION ONCOLOGY BRANCH

NATIONAL CANCER INSTITUTE

OCTOBER 1, 1979 to SEPTEMBER 30, 1980

The Radiation Oncology Branch is in the process of completing its period of transition. There has been an almost complete turnover of staff, both physician, technical, and radiation biology over the past two years.

The three main goals of the Radiation Oncology Branch are the following:

1. Major emphasis on clinical trials of combined modality nature, predominantly collaborative with other clinical branches.
2. Strong radiation biology program with heavy emphasis on basic science and clinical questions of relevance.
3. A training program in radiation therapy, equivalent in stature to the present programs in Medical, Surgical, Pediatric Oncology Branches within the Cancer Institute.

A radiation therapy training program was submitted to the American Board of Radiology and the AMA Residency Review Committee this last year. It was not approved, because of the fact that it was submitted for only a two year program and because of a skewed patient population base present at the Cancer Institute, with inadequate numbers of head and neck and gynecologic oncology patients. A new program is being contemplated and will be submitted soon, probably in conjunction with the military hospitals through the Uniformed School of Health Sciences.

A formal radiation biology program is in the process of getting underway within the ROB. The program is still in an embryonic phases, because of inadequate space, a problem which will not be corrected until our new clinical facility has been opened. Once this is done, our present clinical space will be renovated into laboratory space and the biology program figures to move quickly. During the interval, Dr. James Mitchell and Dr. Elizabeth Travis are organizing tissue culture and radiobiology experiments. Many of these experiments at the present time deal with chemotherapeutic agents, because of problems with radiating devices, (all of which are used clinically) which will be corrected shortly. Long range programs include low dose rate radiation effects, normal tissue tolerance studies, and a large program dealing with radiosensitizing and radioprotecting compounds with which the branch will become intimately involved clinically as well as in the laboratory. Preliminary studies have been going on with human CFUc, through the guidance of Dr. Ross Abrams. These studies have also led to studies of hyperthermia on human CFUc which are also ongoing. Early animal work is concentrating on skin reactions and lung systems, areas in which Dr. Travis is an expert.

Many of the traditional clinical studies of this branch are now terminated. There is no chronic lymphocytic leukemia protocol in existence. Studies of Hodgkin's disease and non-Hodgkin's lymphomas are now collaborative with the Medicine Branch. These studies have been somewhat slow to accrue patients, but it is too soon to tell whether or not this will be a persistent problem.

The study of small cell carcinoma of the lung is now a collaborative venture with the VA Medical Oncology Branch. This collaboration has been predominantly involved with a controlled prospective study of the value of irradiation to the chest in patients with limited disease. Preliminary results suggest that there is a significant benefit to combined modality treatment. However, additional patients are necessary before this conclusion can be reached. New studies going on for advanced oat cell carcinoma will also soon include radiation therapy.

A large part of this past year was involved with recommissioning of our two linear accelerators. This is felt to be necessary and important prior to obtaining new equipment for the new department, when that opens. A number of major problems were identified in the recommissioning, which have all been resolved. Extensive work was undertaken by Dr. Thomas Padikal on the electrons available from the Mevatron 12. This work is culminated in initiating whole skin electron beam treatment for mycosis fungoides, in recent collaboration with the VA.

In addition to the above mentioned collaborative studies, the ROB continues to be actively participating in studies with the Surgery Branch in soft tissue sarcomas, and the Pediatric Oncology Branch in Ewing's sarcoma, rhabdomyosarcoma, and lymphomas. In addition, a new Phase I study of whole body irradiation is planned in conjunction with the VA in multiple myeloma.

Studies that are intrinsic to the ROB have begun this past year. Probably the most important is a controlled study of hypoxic cell sensitizing drugs (misonidazole, intravenously administered) in the treatment of carcinoma of the esophagus. Patients are treated with preoperative irradiation, with half the patients being randomized to receive intravenous misonidazole with each fraction of irradiation. The preliminary data look very encouraging, but the number of patients on study during this first year is still relatively small. Of the first 6 patients who have completed treatment, half of them are doing well without any evidence of disease. The other 3 patients have all suffered acute respiratory failure in the immediate postoperative period. This does not appear to be radiation pneumonitis but conceivably represents an unusual manifestation of combined radiation-surgery interactions, compounded by anesthesia and possibly oxygen toxicity. Carcinoma of the esophagus also offers, in conjunction with the Surgery Branch, an excellent opportunity to do controlled nutritional studies in this patient population, which is frequently malnourished.

The second major group of studies going on the Radiation Oncology Branch is intraoperative radiation. Presently patients are operated on on the 10th floor for carcinoma of the pancreas, carcinoma of the stomach, or retroperi-

toneal sarcomas. Maximum surgery is performed, and the patients are then transported through the hallways and elevators to the Radiation Oncology Branch while under general anesthesia. Once in the ROB, they are taken to the treatment room moved to the treatment couch, and re-opened so that large single doses of electron beam therapy are applied intraoperatively, in conjunction with misonidazole. These studies have just begun and the early results are clearly premature for analysis.

The third major ROB study deals with Stage I and II breast cancer, comparing radical surgery to radical irradiation with preservation of the breast in this disease. Patient accrual has been spotty for this particular randomized study. Nonetheless, over 20 patients have already been accumulated in less than a year since its inception, a figure which is probably acceptable since there was no accrual pattern for such patients previously.

A final ROB study deals with intravenous misonidazole, the hypoxic cell sensitizer, in the treatment of locally unresectable osteogenic sarcoma. At the time of this writing, 3 patients with osteogenic sarcomas of the sacrum have undergone high dose irradiation in conjunction with the radiation sensitizer. None has manifested growth of the local tumor site thus far. Long term followup is obviously important before any conclusion can be made.

Through the efforts of several people, including Dr. Joel Tepper (who has joined our staff this last year) the program for incorporating CT scanning into treatment planning has now become a reality. The vast majority of patients whom we treat are now scanned in the treatment position and the computerized treatment plans are generated, superimposed on the CT sections. This has allowed for extraordinarily sophisticated radiation therapy treatment plans, which we employ on our esophagus patients, our breast patients, and many of the soft tissue sarcomas. These particular problems are all complex in terms of their anatomic relationships, and the usefulness of CT in treatment planning cannot be denied for these particular problems. Ultimately, we hope to be able to incorporate CT scanning with computerized treatment planning through any plane, not just simply a cross sectional plane. We also intend to interface ultrasound into the program and compare it to CT.

A good portion of time has been devoted to the plans for the new radiotherapy treatment facility. This facility has been under construction for some time and has already past its due date. Several problems have led to delays and we hope to have it by January of 1981. As soon as this facility is occupied, our present clinical facility will be renovated into a radiation biology laboratory. One of the major changes that took place this last year was the decision to take the 4th bay in the new department and renovate the space into an intraoperative facility. Surgery will actually be performed in that room, with a radiating device dedicated to the intraoperative program. The major advantages of the intraoperative irradiation would appear to be speed, collaboration with surgeons, direct observation of the precise localization of tumor, and the ability to eliminate critical normal tissues from the usual radiation field. In addition, it offers a superb research opportunity with which to combine radiosensitizing drugs and even hyperthermia in the treatment of intraabdominal

neoplasms, cancers which have always proved to be difficult ones in which to achieve success. It may also prove to be the ideal approach to the treatment of retroperitoneal nodes for pelvic neoplasms.

CONFIDENTIAL SOURCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 00650-25 RO																																	
PERIOD COVERED October 1, 1979 to September 30, 1980																																			
TITLE OF PROJECT (80 characters or less) Service Radiation Therapy																																			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																			
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Allen S. Lichter, M.D.</td> <td style="width: 40%;">Head, Radiation Ther. Section</td> <td style="width: 20%;">ROB, NCI</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Others: Joel Tepper, M.D.</td> <td>Sr. Investigator, Rad. Ther. Sec.</td> <td>ROB, NCI</td> </tr> <tr> <td>James Schwade, M.D.</td> <td>Head, Rad. Biological Section</td> <td>ROB, NCI</td> </tr> <tr> <td>Marilyn Glover, R.N.</td> <td>Clinical Nurse</td> <td>ROB, NCI</td> </tr> <tr> <td>Andrea Zola, R.T.</td> <td>Medical Radiation Technician</td> <td>ROB, NCI</td> </tr> <tr> <td>Sandra Franklin, R.T.</td> <td>" " "</td> <td>ROB, NCI</td> </tr> <tr> <td>Joy Greig, R.T.</td> <td>" " "</td> <td>ROB, NCI</td> </tr> <tr> <td>Barbara Kelly, R.T.</td> <td>" " "</td> <td>ROB, NCI</td> </tr> <tr> <td>Pamela Gurley, R.T.</td> <td>" " "</td> <td>ROB, NCI</td> </tr> <tr> <td>Vicky Iler, R.T.</td> <td>" " "</td> <td>ROB, NCI</td> </tr> </table>			PI: Allen S. Lichter, M.D.	Head, Radiation Ther. Section	ROB, NCI				Others: Joel Tepper, M.D.	Sr. Investigator, Rad. Ther. Sec.	ROB, NCI	James Schwade, M.D.	Head, Rad. Biological Section	ROB, NCI	Marilyn Glover, R.N.	Clinical Nurse	ROB, NCI	Andrea Zola, R.T.	Medical Radiation Technician	ROB, NCI	Sandra Franklin, R.T.	" " "	ROB, NCI	Joy Greig, R.T.	" " "	ROB, NCI	Barbara Kelly, R.T.	" " "	ROB, NCI	Pamela Gurley, R.T.	" " "	ROB, NCI	Vicky Iler, R.T.	" " "	ROB, NCI
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Others: Joel Tepper, M.D.	Sr. Investigator, Rad. Ther. Sec.	ROB, NCI																																	
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Barbara Kelly, R.T.	" " "	ROB, NCI																																	
Pamela Gurley, R.T.	" " "	ROB, NCI																																	
Vicky Iler, R.T.	" " "	ROB, NCI																																	
COOPERATING UNITS (if any) None																																			
LAB/BRANCH Radiation Oncology Branch																																			
SECTION Radiation Therapy Section																																			
INSTITUTE AND LOCATION National Cancer Institute, National Institutes of Health, Bethesda, Maryland																																			
TOTAL MANYEARS: 5	PROFESSIONAL: 2	OTHER: 3																																	
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																																			
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to provide expert radiotherapy, consultation and radiation therapy treatment for Clinical Center patients admitted to services other than the Radiation Oncology Branch of the NCI. Support is given to the Medicine Branch, Surgery Branch, Pediatric Oncology Branch, VA Medical Oncology Branch, Neurosurgical Service, Endocrine Service, and other Federal Hospitals in the area where technical expertise and technical equipment dictate a need for such consultation.																																			

Project Description:

Objectives: To provide consultation and radiation treatment for Clinical Center patients.

Methods: Formal and informal consultation with referring physicians and application of radiation therapy where appropriate with x-rays and electrons in accordance with standard radiation therapy practice as well as modified programs where necessitated by adjuvant concomitant therapies.

Major Findings: There were 487 patients seen in formal consultation and an additional approximately 300 telephone consultations provided "ad hoc" advice on treatment or general information. Approximately 450 patients will be treated in this fiscal year with the majority of these being service patients and the remainder being protocol patients in the Radiation Oncology Branch.

Proposed Course: To continue.

Publications:

Brereton, H. D., Young, R. C., Longo, D. L., Kirkland, L. R., Berard, C. W., Jaffe, E. S., DeVita, V. T., and Johnson, R. E.: A comparison between combination chemotherapy and total body irradiation plus combination chemotherapy in non-Hodgkin's lymphoma. Ca 43:2227-2231, 1979.

Katin, M. J., Brereton, H. D., and Michaelis, L. L.: Surgical repair of esophagobronchial fistula following successful treatment of Hodgkin's disease. Ca 44:1121-1124, 1979.

O'Donnell, J. F., Brereton, H. D., Greco, F. A., Gralnick, H. R., and Johnson, R. E.: Acute nonlymphocytic leukemia and acute myeloproliferative syndrome following radiation therapy for non-Hodgkin's lymphoma and chronic lymphocytic leukemia: clinical studies. Ca 44:1930-1938, 1979.

Glatstein, E.: Radiotherapy in the management of nonseminomatous testicular carcinoma. Ca Treat Rep. 63:1649-1651, 1979.

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Services Nonclinical Irradiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas N. Padikal	Senior Staff Fellow	ROB	NCI
Other:	Vicki Iler	Ther. Rad. Tec. (Dos.)	ROB	NCI
	Frank Harrington	Engin. Tec.	ROB	NCI
	Peter L. Roberson	Staff Fellow	ROB	NCI
	Benedick A. Fraass	Staff Fellow	ROB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

.5

PROFESSIONAL:

.2

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☒ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Provide radiation physics services, space, equipment, and advice on experiments involving radiobiology. Cells, tissue cultures, mice and dogs were irradiated for radiobiology experiments.

Project Description:

Objectives: To provide space, equipment and radiation physics expertise to all researchers at the National Institutes of Health who are involved with radiobiological projects.

Methods Employed: Informal consultation with researchers who use the 250 Kvp x-ray units for radiobiology experiments. Informal training of each researcher in the proper procedure for safety using the x-ray facility. Fabrication of devices to hold animals in specific positions relative to x-ray beam while shielding certain critical organs.

Major Findings: Over 1000 hours of exposure time was reserved for researchers. Materials irradiated included cells, tissue cultures, mice, rats, and dogs. The A.F.F.R.I. 50 MV linear accelerator was again used for a series of dog studies.

Significance to Biomedical Research and the Program of the Institute: Provides essential radiation physics support to ongoing research of the National Cancer Institute and other institutes.

Proposed Course: To be continued. The mid-line dose distribution in mice will be mapped to compare dorsal versus ventral irradiation. The 250 Kvp facility will be restricted to use by those researchers who must have irregular field shapes. Dosimetry techniques, systems, devices and phantoms needed to support the expanding Radiobiology Section, ROB, COP, DCT, NCI will be developed.

Publications: None

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Rad. Ther. Treatment Planning Using Computerized Tomography Interfaced
with a Treatment Planning System.NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Thomas N. Padikal	Senior Staff Fellow	ROB	NCI
Other:	Eli Glatstein	Chief	ROB	NCI
	Joel Tepper	Senior Investigator	ROB	NCI
	D. Syed	Head	CSL	CR
	H. A. Fredrickson	Comp. Systems Analyst	CSL	CR
	Marie Chang	Comp. Specialist	ROB	NCI
	Peter L. Roberson	Staff Fellow	ROB	NCI
	Benedick A. Fraass	Staff Fellow	ROB	NCI

COOPERATING UNITS (if any)

Computer Systems Laboratory, CR, NIH

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Physics and Computer Automation Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda MD 20205

TOTAL YEARS:

3.25

PROFESSIONAL:

3.25

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☒ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project deals with a CT based treatment planning system for clinical use in radiation therapy. A large data base, generated from the teletherapy equipment (Mev. XII and Mev. VI), has been entered into the system and a faithful representation of the radiation field in digital form has been accomplished. External Beam treatment planning has been well-documented; brachytherapy documentation has been completed. Color-coded isodose distributions can be superimposed on selected CT cuts. This has improved the methods of dose delivery. The utilization of the system in routine treatment planning has dramatically increased.

Project Description:

Objectives: To utilize the refined imaging capabilities of a CT scanner in improved radiotherapy treatment planning.

Methods employed: A large and new data base was generated using an RFA-3 radiation field analyzer. The computer parameters have been experimentally adjusted to agree with the measurements.

The Computer systems laboratory has continued their assistance in providing the programming and other needs of the Branch. Patient specific information is generated from the CT scan and intricate treatment planning is done with the aid of color displays that have high spatial resolution. The radiation beams are mathematically modelled using commercially available software. Several features were incorporated here, such as the generation of a Cartesian coordinate system, the normalization of isodoses to a reference point, the ability to display selected isodoses, the continuous dose distribution algorithm and automatic contouring. Currently over 80% of the patients receive planning.

Major Findings: Radiation dose distributions can be electronically superimposed on CT scans. The precision in treatments can be favorably affected by treatment plans.

Significance to Biomedical Research and the Program of the Institute: This system improves the accuracy of treatment planning and the therapeutic ratio and, therefore, minimizes the morbidity to the patient.

Proposed Course: Continue R & D work to further improve the capabilities of the system. The R & D work is to include dose optimization and three-dimensional treatment planning.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 00997-04 R0
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Service Clinical Radiation Physics		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	Thomas N. Padikal Peter L. Roberson Benedick A. Fraass Vicki Iler Frank Harrington	Senior Staff Fellow Staff Fellow Staff Fellow Ther. Rad. Tec. (Dos.) Engin. Tec.
		ROB ROB ROB ROB ROB NCI NCI NCI NCI NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Radiation Oncology Branch		
SECTION Radiation Physics and Computer Automation Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205		
TOTAL MAN-YEARS: 4.0	PROFESSIONAL: 2.25	OTHER: 1.75
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Provide <u>expert consultations in Radiation Physics</u> , <u>radiotherapy treatment planning and calibration services of accelerators</u> for purposes of treatment of clinical center patients admitted for services in the Radiation Oncology Branch of the National Cancer Institute. The Branch now has the capability of treating with a <u>10 MV photon beam</u> .		

Project Description:

Objectives: To provide equipment and physics consultation for the radiation therapy of patients at the National Institutes of Health.

Methods Employed: Linear accelerators were calibrated and maintained to provide accurate radiation delivery for patients undergoing radiation therapy. Computers were employed to generate treatment plans in which beam parameters were varied to determine the optimum for patient care. Informal consultations were held with radiotherapists concerning energy, modality, and configuration of beams.

Major Finding: Dosimetry, treatment planning and treatments were provided for patients undergoing radiation therapy. One accelerator was upgraded to produce a 10 MV photon beam and the other was recommissioned to establish base line data for dosimetric, mechanical, and treatment beam parameters. Detailed equipment specifications were established and contracts negotiated for an accelerator to be installed in the new therapy facility for intraoperative therapy. Sealed source implant services were provided including procurement of sources, dosimetry of sources and establishment of inventory control. Intraoperative electron therapy services continued with studies on the A.F.F.R.I. linear accelerator, design and construction of cones for electrons, and x-ray contamination studies. The x-ray dose distributions of existing accelerators were field mapped and computer encoded into a PDP11/70 computer to provide treatment plan accuracies of 1% on single field treatment plans.

Significance to Biomedical Research and the Program of the Institute: Provides essential radiation physics support for ongoing clinical research programs of the National Cancer Institute and other Institutes with programs in the Clinical Center.

Proposed Course: To continue.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 00998-02 R0																				
PERIOD COVERED October 1, 1979 to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Study of Radiation Sensitizers in Carcinoma of the Esophagus																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">J. Schwade, M.D.</td> <td style="width: 25%;">Acting Hd, Radiobiology</td> <td style="width: 10%;">ROB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td></td> <td></td> <td style="text-align: center;">Section</td> <td></td> <td></td> </tr> <tr> <td>Other:</td> <td>E. Glatstein, M.D.</td> <td>Chief</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td colspan="2">Other Staff of the Radiation Oncology Branch</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI:	J. Schwade, M.D.	Acting Hd, Radiobiology	ROB	NCI			Section			Other:	E. Glatstein, M.D.	Chief	ROB	NCI		Other Staff of the Radiation Oncology Branch		ROB	NCI
PI:	J. Schwade, M.D.	Acting Hd, Radiobiology	ROB	NCI																		
		Section																				
Other:	E. Glatstein, M.D.	Chief	ROB	NCI																		
	Other Staff of the Radiation Oncology Branch		ROB	NCI																		
COOPERATING UNITS (if any) Surgery Branch, COP, DCT, NCI																						
LAB/BRANCH Radiation Oncology Branch																						
SECTION Clinical Radiation Therapy Section																						
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205																						
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																				
3.0	2.0	1.0																				
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study radiation sensitizing compounds and their ability to increase the effect of radiation in sterilizing neoplastic cells. The plan is to take patients who have carcinoma of the esophagus that appears to be clinically confined to the mediastinum and give all patients preoperative irradiation. Half the patients will receive misonidazole, a hypoxic cell sensitizer, administered by an intravenous method. Following completion of the radiotherapy, surgery will be performed and survival, freedom from lapse, and tumor viability within the pathologic specimen will all be evaluated. In addition, these patients, who are often frequently cachectic, will serve as the basis for controlled studies of total parenteral nutrition.																						

Project Description:

Objectives: By assessing the survival, freedom from lapse, and tumor viability within the pathologic specimen, we hope to be able to determine whether or not misonidazole is effective in augmenting radiation effects in patients with carcinoma of the esophagus. If this compound does appear to increase radiation effectiveness, then further work in radiosensitizing compounds is clearly warranted.

Methods Employed: Patients with previously untreated carcinoma of the esophagus confined clinically to the mediastinum will be accessioned and treated with preoperative radiation. Half the patients will receive on a randomized basis intravenously administered misonidazole with each fraction of irradiation.

Major Findings: Six patients have completed therapy, and a seventh is currently undergoing therapy. Of the patients who have completed therapy, there have been three pulmonary deaths, which have led to some modifications of the treatment. The remaining three patients are alive and free of disease, the longest eight months post completion of therapy.

Significance of the Research Program to the Institute: Radiation sensitizing drugs may represent an avenue to increase effectiveness of therapy by augmenting the effects of an existing modality, radiation. This study represents an attempt to evaluate the efficacy of the drug currently available and thought to be most effective, misonidazole.

Proposed Course: Patient accrual continues.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03424-03 RO
PERIOD COVERED August 1, 1979 to June 30, 1980		
TITLE OF PROJECT (80 characters or less) Whole Body Hyperthermia combined with Chemotherapy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Joan M. Carlson Bull, M.D., ROB, NCI Others: Rosalie Smith, R.N., ROB, NCI David E. Lees, M.D., Anesthesiology Service, CC William Schuette, B.E.E., BEIB, CC Michael Dubois, M.D., Anesthesiology Service, CC		
COOPERATING UNITS (if any) Biomedical Engineering and Instrumentation Branch, DRS Division of Anesthesia, CC		
LAB/BRANCH Radiation Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION National Cancer Institute, National Institutes of Health, Bethesda, Md.		
TOTAL MANYEARS: 5	PROFESSIONAL: 5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study the effectiveness and the toxicity of combining whole body hyperthermia with several chemotherapeutic agents in patients with metastatic melanoma and other patients with advanced sarcoma.		

Project Description:

Objectives: Based on prior phase I trials of whole body hyperthermia alone and whole body hyperthermia plus adriamycin and with the observation that patients with melanoma had responses to heat alone it was decided to look at the effect of the nitrosourea methyl-CCNU in an inpatient with metastatic melanoma, and the combination of adriamycin and cytoxan in patients with advanced sarcoma. In vitro and in vivo data exists for synergistic increase in Cyto-toxicity of the nitrosoureas, and adriamycin with hyperthermia.

Methods Employed: Patients with metastatic melanoma under the age of 65 years with no metastases to the brain, and with normal cardiovascular systems as screened by history, physical examination and EKG's, were subjected on day 1 to whole body hyperthermia induced by a micro processor controlled high flow low pressure water suit to temperatures of 41.8°C for two hours. These patients underwent physiologic studies including intrapulmonary artery catheterization, brain EEG's and continuous monitoring with esophageal and rectal temperatures in addition to ongoing continuous EKG, blood pressure and arterial pressures as well as arterial blood gases. The tolerance to this procedure was observed. On Day 8-12 the patient underwent whole body hyperthermia again. At the time of reaching temperature, (41.8°C) which occurs 1 1/2 to 3 hours after beginning the procedure, the patients received an oral dose of methyl-CCNU beginning at 75 mg/m^2 and escalating every two patients up to a full dose of 250 mg/m^2 . After receiving the chemotherapy the patient was again heated for a total period of 2 hours at temperature.

Major Findings: Twelve patients with melanoma were treated on this protocol. Toxicities were 0/12 granulocytopenia, 0/12 thrombocytopenia, 0/12 alopecia, 2 of 12 peripheral neuropathy, 5 of 12 pressure burns, 0 of 12 post-procedural fevers, 8 of 12 emesis, 4 of 12 diarrhea, 2 of 12 patients experienced herpetic blisters. Because of the lack of myelosuppression, it was determined there was no active drug reaching either bone marrow or tumor sites. Despite this, of the 12 patients, 5 experienced progressive disease, 1 had stable disease, 2 patients had a mixed response, 4 patients had an objective response.

Because of lack of myelosuppression the nitrosourea was changed to BCNU an, I.V. preparation of nitrosourea. 13 patients were treated on this protocol with chemotherapy doses from 175 mg/m^2 and escalated every 2 patients to a total dose of 225 mg/m^2 .

Results: Thirteen patients received this combination of whole body hyperthermia and BCNU. Toxicities were 0 of 13 patients experienced alopecia, 3 of 13 patients developed neutropenia of less than 1000 cells/mm^3 and 5 of 13 patients experienced thrombocytopenia of less than $15000\text{ platelets/mm}^3$. Five of 13 patients experienced post-procedural fevers, 1 of 13 patients developed peripheral neuropathy, 3 of 13 patients experienced

pressure burns, 8 of 13 patients experienced emesis, 4 of 13 patients had diarrhea and 2 of 13 patients had herpetic blisters. There were 8 patients that had progressive disease, 4 patients experienced subjective responses and 1 patient developed a complete response which is duration 65+ weeks.

One patient was treated with the combination of adriamycin and cytoxan with the procedure as described above. The patient was treated on day 1 with heat alone, on day 8-12 with adriamycin 50 mg/m² at time of maximum temperature. Then the patient was heated for another 2 hours. Four hours after the end of the heat treatment, cytoxan 1 gm/m² was administered I.V.. One patient had a short objective response lasting 2 months and the unusual toxicity observed was a seeming lymphatic obstruction of the upper torso that was transient.

Significance: Synergistic activity of the combination of BCNU plus whole body hyperthermia and metastatic melanoma and the myelotoxicity of this combined regimen limited further therapy in the four objective responding patients. There was an objective response of the patient receiving adriamycin-cytosine but this was short lived following the toxicity of lymphatic obstruction that did not allow for re-application of the heat procedure.

Proposed Course: We will continue the protocols of nitrosourea plus heat in patients with metastatic melanoma.

Publications:

1. Lees, D.E., Kim, Y.D., Bull, J.M., Whang-peng, J., Schuette, W., Smith, R., MacNamara, T. E.: Anesthetic management of whole body hyperthermia for the treatment of cancer. *Anesthesia*, 1444, 1980.
2. Editor: Storm, K.: Chapter in Textbook Hyperthermia in Cancer Therapy, G. K. Hall Boston "History Rationale Physiology and Toxicity of Systemic Hyperthermia", Bull, J., (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06310-01 R0
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Surgery versus Radiation Therapy in Treatment of Primary Breast Cancer		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Allen S. Lichter, M.D. , Head, Radiation Therapy Section, ROB, NCI Others: Other Staff within the Radiation Oncology Branch, NCI.		
COOPERATING UNITS (if any) Surgery Branch, NCI		
LAB/BRANCH Radiation Oncology Branch		
SECTION Radiation Therapy Section		
INSTITUTE AND LOCATION National Cancer Institute, National Institutes of Health, Bethesda, Maryland		
TOTAL MANYEARS: 5	PROFESSIONAL: 2	OTHER: 3
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study whether techniques of treatment that preserve the breast (lumpectomy followed by radiation therapy) provide equal survival opportunity when compared to women treated with standard surgical techniques (mastectomy) for primary breast cancer. After a workup confirms localized disease the patients are randomized to treatment with either mastectomy or lumpectomy plus radiation therapy. Both groups have axillary node dissections and are treated with chemotherapy should the nodes be positive.		

Project Description:

Objectives: Survival and recurrence figures are comparable for the two treatments, it should be far more acceptable for women to be treated with less than radical surgical procedures for localized breast cancer. The cosmetic result of localized treatment will be carefully evaluated. The psychological, sexual, and sociological impact of mastectomy versus lumpectomy will be noted. The ability to combine radiation therapy with aggressive chemotherapy in node positive patients will also be assessed.

Methods Employed: Patients with previously untreated carcinoma of the breast clinically and radiographically confined to the breast and axillary lymph nodes will be accessioned into the study. They will be randomized to have treatment with lumpectomy and radiation therapy versus mastectomy. Patients with positive axillary lymph nodes will receive chemotherapy.

Major Findings: This study has been active for 9 months. Currently 19 patients are enrolled and it is far too early to assess results.

Proposed Course: Patient accrual is underway and will continue.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06311-01 RO
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Real-Time Radiotherapy Treatment Monitor		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Thomas N. Padikal PI: Benedick A. Fraass Other: Peter L. Roberson	Senior Staff Fellow Staff Fellow Staff Fellow	ROB NCI ROB NCI ROB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Radiation Oncology Branch		
SECTION Radiation Physics and Computer Automation Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of the project is to develop a <u>real-time monitor for radiation treatments</u> . Although routine quality assurance is the immediate aim, continued development will make feasible many projects which rely on real-time patient dose monitoring.		

Project Description:

Objectives: To design and develop a real-time monitoring device for radiotherapy treatments of patients.

Methods Employed: A two-dimensional array of radiation-sensing diodes is used to monitor the radiation which is transmitted through the patient during a treatment. From knowledge of the transmitted intensity, patient dose information is obtained. A microcomputer - based system is used to accumulate and analyze the data from the diode array.

Major Findings: Initial hardware interfacing, computer programming, and diode selection have been accomplished. The system has been tested and found to be quite promising. Optimization of the many factors affecting system performance is now underway.

Significance to Biomedical Research and the Program of the Institute: This project is expected to improve the quality of radiotherapy treatment in the Radiation Oncology Branch. Further development will lead to innovative and more precise types of treatments.

Proposed Course: To be continued. The present data acquisition system will be developed and refined. Design of the system to be used for automatic monitoring and recording of patient treatments will proceed. Investigation into the use of the diode array system for beam symmetry, quality, and calibration checks, compensating filter design, dynamic radiotherapy treatments, quality control, and real-time treatment analysis will continue.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06312-01 R0																				
PERIOD COVERED October 1, 1979 to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Physical Aspects of Total Skin Electron Irradiation.																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Thomas N. Padikal</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 10%;">ROB</td> <td style="width: 20%;">NCI</td> </tr> <tr> <td>Other: Eli Glatstein</td> <td>Chief</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td>Benedick A. Fraass</td> <td>Staff Fellow</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td>Peter L. Roberson</td> <td>Staff Fellow</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td>Vicki Iler</td> <td>Rad. Ther. Tec. (Dos.)</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI: Thomas N. Padikal	Senior Staff Fellow	ROB	NCI	Other: Eli Glatstein	Chief	ROB	NCI	Benedick A. Fraass	Staff Fellow	ROB	NCI	Peter L. Roberson	Staff Fellow	ROB	NCI	Vicki Iler	Rad. Ther. Tec. (Dos.)	ROB	NCI
PI: Thomas N. Padikal	Senior Staff Fellow	ROB	NCI																			
Other: Eli Glatstein	Chief	ROB	NCI																			
Benedick A. Fraass	Staff Fellow	ROB	NCI																			
Peter L. Roberson	Staff Fellow	ROB	NCI																			
Vicki Iler	Rad. Ther. Tec. (Dos.)	ROB	NCI																			
COOPERATING UNITS (if any) VA Medicine Branch																						
LAB/BRANCH <u>Radiation Oncology Branch</u>																						
SECTION <u>Radiation Physics and Computer Automation Section</u>																						
INSTITUTE AND LOCATION <u>NCI, NIH, Bethesda, MD 20205</u>																						
TOTAL MANYEARS: <u>1.0</u>	PROFESSIONAL: <u>.9</u>	OTHER: <u>.1</u>																				
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) The Mev XII linear accelerator has been modified for use in total skin electron irradiation, such as <u>mycosis fungoides</u> . Dosimetric features have been ascertained and <u>treatment</u> of mycosis fungoides patients has been initiated.																						

Project Description:

Objectives: To provide radiation physics services, equipment and consultation for treatment of mycosis fungoides patients.

Methods Employed: The MeV XII linear accelerator has been successfully modified to produce a beam suitable for whole skin treatment. Patients are currently undergoing treatment for mycosis fungoides. A dual field is used to treat each position of the patient for a total of six positions. The dosimetric features of the beam have been well documented. Thermoluminescent dosimetry (TLD) measurements are used for ongoing quality assurance. Eye shields are used to protect the eye. More research and development is required to refine the method.

Major Findings: Patients suffering from mycosis fungoides can be treated using the MeV XII linear accelerator. More refined treatment techniques are indicated. TLD data have been and are being accumulated.

Significance to Biomedical Research and the Program of the Institute: This program is conducted in cooperation with the Medicine Branch of the VA Hospital.

Proposed Course: To be continued. More R & D work is contemplated for improved technique of irradiation.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06313-01 RO																														
PERIOD COVERED October 1, 1979 to September 30, 1980																																
TITLE OF PROJECT (80 characters or less) Dose to Lung and Opposite Breast Vs. Technique for Primary Breast Irradiation																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																																
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">P .L. Roberson</td> <td style="width: 40%;">Staff Fellow</td> <td style="width: 10%;">ROB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>A. S. Lichter</td> <td>Head, Clin. Rad. Ther. Sec.</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>H. A. Fredrickson</td> <td>Comp. Systems Analyst</td> <td>DCRT</td> <td>NIH</td> </tr> <tr> <td></td> <td>T. N. Padikal</td> <td>Senior Staff Fellow</td> <td>ROB</td> <td>NIH</td> </tr> <tr> <td></td> <td>B. A. Kelley</td> <td>Chief Ther. Tec.</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>V. L. Iler</td> <td>Ther. Rad. Tec. (Dos.)</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI:	P .L. Roberson	Staff Fellow	ROB	NCI	Other:	A. S. Lichter	Head, Clin. Rad. Ther. Sec.	ROB	NCI		H. A. Fredrickson	Comp. Systems Analyst	DCRT	NIH		T. N. Padikal	Senior Staff Fellow	ROB	NIH		B. A. Kelley	Chief Ther. Tec.	ROB	NCI		V. L. Iler	Ther. Rad. Tec. (Dos.)	ROB	NCI
PI:	P .L. Roberson	Staff Fellow	ROB	NCI																												
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	B. A. Kelley	Chief Ther. Tec.	ROB	NCI																												
	V. L. Iler	Ther. Rad. Tec. (Dos.)	ROB	NCI																												
COOPERATING UNITS (if any) Computer Systems Laboratory, DCRT, NIH																																
LAB/BRANCH Radiation Oncology Branch																																
SECTION Radiation Physics and Computer Automation Section																																
INSTITUTE AND LOCATION NCI, NIH, Bethesda MD 20205																																
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">0.2</td> <td style="text-align: center;">0.15</td> <td style="text-align: center;">.05</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	0.2	0.15	.05																								
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SUMMARY OF WORK (200 words or less - underline keywords) Treatment-planning techniques are investigated which maximize the treatment of areas at risk while minimizing dose to critical structures for primary breast irradiation. When the high-dose volume is increased to include the internal mammary chain (IMC), dose to the lung and opposite breast increase. The finite depth of penetration of an electron field can be used to treat the IMC without a large increase in dose to lung and opposite breast.																																

Project Description:

Objectives: To quantify dose to critical structures (lung, opposite breast) as a function of treatment technique. Of particular interest is comparing techniques which include the internal mammary chain (IMC) within the high-dose volume.

Methods Employed: Dose distributions for both conventional and unconventional treatment techniques were planned over CT scans. Dose to the lung and to the opposite breast were recorded. Dose distributions were verified by phantom measurements and by measurement of surface dose for patients under treatment.

Major Findings: The majority of the techniques have both advantages and disadvantages: the high-dose volume can be increased at the expense of increased dose to critical structures. The optimum technique investigated is combining shallow photon field tangents with a separate IMC electron field.

Significance: An improvement in therapeutic ratio (dose to area at risk/dose to normal tissue) will reduce possible complications of treatment, allow better coverage of the tumor volume by the high-dose region, or both.

Proposed Course: (1) Dose to critical structures will be calculated and measured for many patients in order to study variations with patient shape. (2) Technical details will be studied for the implementation of the combined photon and electron field treatment technique.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06314-01 RO								
PERIOD COVERED October 1, 1979 to September 30, 1980										
TITLE OF PROJECT (80 characters or less) Quantitation of Teletherapy Beam Asymmetry for All Gantry Angles										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT										
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Thomas N. Padikal</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 15%;">ROB</td> <td style="width: 19%;">NCI</td> </tr> <tr> <td>Other: Peter L. Roberson</td> <td>Staff Fellow</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI: Thomas N. Padikal	Senior Staff Fellow	ROB	NCI	Other: Peter L. Roberson	Staff Fellow	ROB	NCI
PI: Thomas N. Padikal	Senior Staff Fellow	ROB	NCI							
Other: Peter L. Roberson	Staff Fellow	ROB	NCI							
COOPERATING UNITS (if any)										
LAB/BRANCH Radiation Oncology Branch										
SECTION Radiation Physics and Computer Automation Section										
INSTITUTE AND LOCATION NCI, NIH, Bethesda MD 20205										
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: .0								
CHECK APPROPRIATE BOX(ES)										
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER										
☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords)										
A simple and efficient means of evaluating <u>teletherapy beam asymmetry</u> was developed for both photon and electron fields. Radiation detectors placed at the periphery of the largest radiation fields monitored the dose rate while the accelerator gantry was rotated through 360°. The ratio of the readings was recorded. This technique can be a valuable part of the <u>acceptance testing</u> procedure of teletherapy equipment.										

Project Description:

Objectives: To develop a simple and efficient means of evaluating the stability and symmetry of photon and electron teletherapy beams at all gantry angles. This is a diagnostic procedure to aid in the detection of unacceptable beam behavior.

Methods Employed: The principle concept is to monitor the dose-rate output at several positions as the accelerator gantry is rotated through 360°. Radiation detectors (diodes or ion chambers) are mounted on the gantry. Dose rates at the beam periphery and central axis are recorded and compared as a function of angle.

Major Findings: Teletherapy beam asymmetry can be measured for all gantry angles. The quantitated beam symmetry displayed characteristics peculiar to each accelerator tested.

Significance to Biomedical Research and the Program of the Institute: The development of practical, more stringent diagnostic tests of teletherapy equipment will ultimately improve the reliability of patient treatments. Also, early detection of beam asymmetry will save time and effort during acceptance-testing procedures.

Proposed Course: Project Complete.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06315-01 RO
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PERIOD COVERED
October 1, 1979 through March 1, 1980

TITLE OF PROJECT (80 characters or less)

Phase I Study of Intra-operative Radiation Therapy for Abdominal and Pelvic Malignancies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joel Tepper, M.D. Senior Investigator ROB, NCI
William Sindelar, M.D. Senior Investigator SB, NCI
Eli Glatstein, M.D. Chief, ROB ROB, NCI
Steven Rosenberg, M.D. Chief, SB SB, NCI
Senior Staff Radiation Oncology Branch ROB, NCI
Senior Staff Surgery Branch SB, NCI

COOPERATING UNITS (if any)

Surgery Branch

LAB/BRANCH

Radiation Oncology Branch

SECTION

Radiation Therapy Section

INSTITUTE AND LOCATION

National Cancer Institute, National Institutes of Health, Bethesda, Md. 20205

TOTAL MANYEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To study the ability to combine intra-operative radiation therapy with radical surgery for various abdominal and pelvic malignancies.

The plan of the Phase I study is to take patients who had conventionally unresectable or marginal resectable intra-abdominal and pelvic carcinomas and to perform a maximal surgical resection. This was followed by a single dose of 2000 rad of intra-operative radiation therapy, in order to improve the local control and thus cure rate of these tumors. Six patients have been irradiated to date. The tolerance of this radiation therapy combined with radical surgery seems to be acceptable. There have been no major complications attributable to the radiation therapy. No statement can be made to date as to the efficacy of this treatment with regard to local control of tumor.

Project Description:

Objectives: By assessing the acute and long term tolerance of patients who have received intra-operative radiation therapy when combined with radical surgery, we plan on evaluating the feasibility of formal randomized trials of intraoperative radiation therapy for various abdominal and pelvic malignancies. If the technique is successful, this could have a major impact on local tumor control.

Methods Employed: Patients with intra-abdominal and pelvic tumors which were marginally resectable by surgery alone had a maximal surgical resection performed. These patients then received 2000 rad of 11 MeV electrons in the radiation therapy department to the area which was at risk for tumor involvement. The patients were transported to the radiation therapy department and intra-operative radiation therapy was carried out while the patient was still under general anesthesia. The patients also received an hypoxic cell sensitizer, misonidazole, with the intra-operative radiation therapy.

Major Findings: The acute tolerance to the intra-operative radiation therapy has been quite good. There have been no major complications secondary to the intra-operative radiation therapy, although there have been some minor transient post-operative problems. It thus appears that it is feasible to combine intra-operative radiation therapy with radical surgery.

Significance to the research program of the institute: Intra-operative radiation therapy has a great deal of appeal as a potential method of delivering a higher biological dose to the tumor while delivering a lesser dose to the surrounding normal structures. Conventional radiation therapy in the abdomen is often limited by the dose which can be delivered to the normal tissue such as liver, kidney, small bowel, stomach, and large bowel. With intra-operative radiation therapy, these structures can be physically removed from the radiation beam. Intra-operative radiation therapy may then allow a higher dose of radiation therapy to be delivered, and thus produce a higher level of local control and cure than conventional techniques. As it appears that radical surgery can be combined with radiation therapy, it would appear feasible to proceed with further studies along this line.

Proposed Course: The Phase I Study has been completed with the conclusion that this is a feasible approach. We have started randomized studies to evaluate the efficacy of this technique for carcinomas of the pancreas, stomach and retroperitoneal soft tissues sarcomas.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06316-01 R0															
PERIOD COVERED October 1979 through September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Study of Normal Tissue Tolerance for Intra-operative Radiation Therapy in Animals																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Joel Tepper, M.D.</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">ROB, NCI</td> </tr> <tr> <td>Others: William Sindelar, M.D.</td> <td>Senior Investigator</td> <td>SB, NCI</td> </tr> <tr> <td>Elizabeth Travis, Ph.D.</td> <td>Senior Investigator</td> <td>ROB, NCI</td> </tr> <tr> <td>R. Terrill, M.D.</td> <td>Senior Investigator</td> <td>SB, NCI</td> </tr> <tr> <td>Thomas Padikal, Ph.D.</td> <td>Senior Staff Fellow</td> <td>ROB, NCI</td> </tr> </table> Staff Armed Forces Radiobiologic Research Institute, Bethesda, Maryland			PI: Joel Tepper, M.D.	Senior Investigator	ROB, NCI	Others: William Sindelar, M.D.	Senior Investigator	SB, NCI	Elizabeth Travis, Ph.D.	Senior Investigator	ROB, NCI	R. Terrill, M.D.	Senior Investigator	SB, NCI	Thomas Padikal, Ph.D.	Senior Staff Fellow	ROB, NCI
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COOPERATING UNITS (if any) Surgery Branch																	
LAB/BRANCH Radiation Oncology Branch																	
SECTION Radiobiology Section																	
INSTITUTE AND LOCATION NIH, NCI, Bethesda, Maryland 20205																	
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:															
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CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) HUMAN SUBJECTS</td> <td>☐ (b) HUMAN TISSUES</td> <td>☒ (c) NEITHER</td> </tr> <tr> <td>☐ (a1) MINDS</td> <td>☐ (a2) INTERVIEWS</td> <td></td> </tr> </table>			☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER	☐ (a1) MINDS	☐ (a2) INTERVIEWS										
☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER															
☐ (a1) MINDS	☐ (a2) INTERVIEWS																
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study the <u>tolerance of normal tissues</u> in dog to intra-operative radiation therapy. This project is being done to be able to determine <u>tolerance levels</u>, and organs likely to be at risk from intra-operative radiation therapy when given for human abdominal neoplasms. Canine abdomens have been opened and then given single large doses of radiation to a treatment volume similar to which would be performed in humans. Some animals had <u>vascular anastomoses</u> irradiated. The animals were then followed to determine sites of potential radiation damage and tolerance dose levels.																	

Project Description:

Objectives: By evaluating the acute and long term tolerance of animals to intra-operative radiation therapy delivered to intra-abdominal fields, we hope to be able to determine which intra-abdominal sites are at risk for radiation damage, and what the tolerance level is for this radiation damage. This will give us valuable information and allow us to better design trials for intra-operative radiation therapy in humans.

Methods employed: One year old dogs were placed under general anesthesia and then had a surgical exploration of the abdomen. The small bowel and other mobile abdominal structures were moved out of the way, and radiation therapy was delivered to a field extending from the level of the diaphragm to the bifurcation of the aorta with the intent of irradiating the retro-peritoneal soft tissues and the large abdominal vasculature. Some of the dogs were also irradiated to aortic anastomoses and some had a blind loop of duodenum irradiated so as to evaluate the tolerance of these structures. Animals were followed clinically, radiographically and in some instances by autopsy to evaluate the acute and long term effects of this radiation therapy.

Major Findings: The acute tolerance and the tolerance out to one year of animals who have received 2000-3000-4000 rad seems to be quite acceptable. There appears to be greater fibrosis and potential damage to retro-peritoneal structures at higher doses. Ureters appear to be especially sensitive to high single doses of irradiation. There is significant number of complications from intra-operative radiation therapy delivered to anastomotic sites at 4500 rad. Preliminary results indicate that doses of 2000-3000 rad would be well tolerated to vascular anastomoses. The tolerance of duodenal stumps could not be adequately evaluated as these animals experienced acute intussusceptions. This finding, however, is also significant.

Significance:

Intra-operative radiation therapy is a technique of potentially broad application in cancer therapy. Conventional radiation therapy is limited by the tolerance of normal structures in the abdomen. With intra-operative radiation therapy one can move these normal structures out of the radiation field, which may result in increased cure rate secondary to the ability to deliver a higher dose to the tumor volume while not irradiating the normal structures. Determining tolerance levels in structures likely to be at risk in canine model is extremely important to help control the direction of the clinical studies in humans.

Proposed Course: We will continue to evaluate the animals which have already been irradiated, and will also irradiate certain other sites which are of specific interest.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 CM 06317-01 R0	
PERIOD COVERED October 1, 1979 to September 30, 1980					
TITLE OF PROJECT (80 characters or less) Toxicologic and Pharmacokinetic Studies of Misonidazole					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:		J. Schwade, M.D.		Acting Hd, Radiobiology	ROB NCI
				Section	
Other:		E. Glatstein, M.D.		Chief	ROB NCI
		Other Staff of the Radiation Oncology Branch			ROB NCI
COOPERATING UNITS (if any) Laboratory of Chemical Pharmacology					
LAB/BRANCH Radiation Oncology Branch					
SECTION Radiation Therapy Section					
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205					
TOTAL MANYEARS: 3.0		PROFESSIONAL: 2.0		OTHER: 1.0	
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) In order to develop more effective radiation sensitizers, as well as more intelligently utilize those currently available, information must be gained regarding the toxicity of current compounds, as well as their pharmacology. With regards to the latter point, studies regarding the modification of the pharmacology may lead to the ability to use the currently available compounds, most notably misonidazole, with less toxicity.					

Project Description:

Objectives: Pharmacokinetic parameters, in plasma, normal tissues and tumors is to be studied.

Methods Employed: Patients have received twice weekly doses of misonidazole intravenously noted to determine base line pharmacokinetic parameters and toxicity, and are currently receiving these drugs with compounds which will modify the pharmacology. Currently, patients are being pre-treated with phenytoin (dilantin) in order to shorten the half-life and decrease the effects of peripheral neuropathy.

Major Findings: Elucidation of the pharmacokinetic parameters of misonidazole in the intravenous form have been determined, as well as maximum tolerated dose in a twice weekly fractionation ($1.5 \text{ gm/m}^2 \text{ 2X/wk} \times 5 \text{ wk}$).

Significance to the Research Program of the Institute: Radiosensitizing drugs may allow more effective use of an existing modality of treatment, radiation therapy, by augmenting the effects of radiation killing in a select population of tumor cells, while having little effect on normal tissue. The Radiation Oncology Branch is the only group in the country currently investigating misonidazole in an intravenous formulation.

Proposed Course: Patient accrual continues at a 2 gm/m^2 twice weekly level with pretreatment with phenytoin. It is planned to continue studies with other radiation sensitizing drugs as they become available.

Publications:

Strong, J.M., Schwade, J.G., Shoemaker, D. and Gangji, D.: Misonidazole dose and tumor level relationship: effects of individual variation on rate of misonidazole metabolism and absorption from the gastrointestinal tract. (Presented at CROS Radiosensitizer/Radioprotector Conference, Key Biscayne, October, 1979, manuscript in press) Clinical Trials.

Schwade, J.G., Strong, J.M. and Gangji, D.: IV misonidazole (NSC 261037)--report of initial clinical experience. (Presented at CROS Radiosensitizer/Radioprotector Conference, Key Biscayne, October, 1979, manuscript in press) Clinical Trials.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06318-01 R0																				
PERIOD COVERED October 1, 1979 to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Locally Unresectable Osteogenic Sarcomas																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Eli Glatstein, M.D.</td> <td style="width: 30%;">Chief</td> <td style="width: 10%;">ROB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>Allen S. Lichter, M.D.</td> <td>Head, Clin Rad Ther Sec</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>James G. Schwade, M.D.</td> <td>Acting Head, Radiobiology</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Joel E. Tepper, M.D.</td> <td>Senior Investigator</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI:	Eli Glatstein, M.D.	Chief	ROB	NCI	Other:	Allen S. Lichter, M.D.	Head, Clin Rad Ther Sec	ROB	NCI		James G. Schwade, M.D.	Acting Head, Radiobiology	ROB	NCI		Joel E. Tepper, M.D.	Senior Investigator	ROB	NCI
PI:	Eli Glatstein, M.D.	Chief	ROB	NCI																		
Other:	Allen S. Lichter, M.D.	Head, Clin Rad Ther Sec	ROB	NCI																		
	James G. Schwade, M.D.	Acting Head, Radiobiology	ROB	NCI																		
	Joel E. Tepper, M.D.	Senior Investigator	ROB	NCI																		
COOPERATING UNITS (if any) Surgery Branch, DCT, NCI																						
LAB/BRANCH Radiation Oncology Branch																						
SECTION Clinical Radiation Therapy Section																						
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205																						
TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5																				
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study the hypoxic cell radiosensitizer misonidazole in conjunction with radiation therapy in the treatment of locally unresectable osteogenic sarcoma and chondrosarcoma. Patients who have a local neoplasm for which no radical surgery can be successfully undertaken will be treated with fractionation radiation therapy in conjunction with intravenously administered misonidazole. Because of the small number of patients who are appropriate for such a study, this will be a one armed study. Following completion of radiation therapy, combination chemotherapy will be administered to patients with osteogenic sarcoma with high grade neoplasms. Following completion of all treatment, survival, freedom from relapse, and complications of treatment will be carefully assessed.																						

Project Description:

Objectives: By assessing the survival, freedom from relapse, and complications of radiation therapy with intravenously administered misonidazole, we hope to be able to determine whether or not such treatment is appropriate for these neoplasms, which have here before been considered "radioresistant". Should the study prove that such treatment is effective, then such treatment could be contemplated for other osteogenic sarcoma patients.

Methods Employed: Patients with locally unresectable osteogenic sarcoma or chondrosarcoma, without overt metastatic disease, will be treated with radiation therapy in conjunction with intravenously administered misonidazole.

Major Findings: Three patients have been entered on the study. In all three, local regrowth of tumor has not yet been seen. Tumor mass has not overtly regressed, but the bony matrix in all 3 patients appear to increase density. One patient remains apparently free of all disease. Another patient has had dissemination; this patient underwent an attempted "debulking" resection prior to radiation therapy. The third patient remains clinically free of disease but has suffered radiation injury to the bowel and ureter.

Significance to the Research Program of the Institute: Radiation sensitizing compounds represent a new avenue of investigation. These compounds are electron affinic and appear to mimic the effects of oxygen, and therefore appear to have the specific ability to make hypoxic cells within a tumor more responsive to radiation killing. Thus, these compounds take a known effect (i.e., radiation killing) and appear to be able to magnify it. If these compounds prove to be effective in humans, then a new direction for clinical investigation in solid tumors will clearly be warranted. Moreover, if these compounds prove to be effective, then the need to invest large sums of money in research directions such as high L.E.T. radiation may be circumvented.

Proposed Course: Patient accrual is underway.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06319-01 RO												
PERIOD COVERED October 1, 1979 to September 30, 1980														
TITLE OF PROJECT (80 characters or less) The use of prematurely condensed chromosomes (PCC) in the biological dosimetry of ionizing radiation.														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: James B. Mitchell, Ph.D.</td> <td style="width: 30%;">Radiation Biologist</td> <td style="width: 10%;">ROB</td> <td style="width: 20%;">NCI</td> </tr> <tr> <td>Other: Nirmolini Soares, M.S.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td>Thomas Bryant, B.S.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI: James B. Mitchell, Ph.D.	Radiation Biologist	ROB	NCI	Other: Nirmolini Soares, M.S.	Biologist	ROB	NCI	Thomas Bryant, B.S.	Biologist	ROB	NCI
PI: James B. Mitchell, Ph.D.	Radiation Biologist	ROB	NCI											
Other: Nirmolini Soares, M.S.	Biologist	ROB	NCI											
Thomas Bryant, B.S.	Biologist	ROB	NCI											
COOPERATING UNITS (if any) None														
LAB/BRANCH Radiation Oncology Branch														
SECTION Radiobiology														
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205														
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to determine if the use of <u>premature chromosome condensation (PCC) technique</u> will improve the resolution of the lymphocyte biological dosimeter system for low total <u>doses of radiation</u> (<10 rad). With the PCC technique, <u>chromosomal damage (gross breaks in chromosomes)</u> of interphase cells can be studied immediately following radiation exposure. Assays will be made before the cells have had time to repair many of the initial breaks, thereby increasing the number of breaks counted as opposed to counting aberrations conventionally 24-48 hours after exposure in metaphase I and II.														

Project Description:

Objectives: By scoring radiation damage in chromosomes (gross breaks) immediately following the exposure, we will construct radiation dose response curves which may provide greater resolution in the low dose region (1-10 rad). If this technique does provide greater resolution to the lymphocyte biological dosimeter system, then the determination of small radiation doses to accidentally exposed persons could be done with a considerable amount of confidence.

Methods Employed: Blood lymphocytes will be irradiated with graded doses of gamma photons and fused immediately with mitotic inducer cells. Slight modifications of the Rao and Johnson PCC technique will be used. Gross breaks in whole G1 PCC's chromosomes will be scored.

Major Findings: The study is in preliminary stages and the results are presently not available for assessment.

Significance to the Research Program of the Institute: Determination of low doses of radiation received by persons accidentally exposed to radiation has been an issue of concern over the past 30 years. More precise methods of accessing low doses of radiation would be of value not only for accidental diagnostic exposure but also for environmental exposures to the general population.

In addition, these studies should provide better understanding as to the nature of chromosome breakage and repair.

Proposed Course: Project is currently underway.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06320-01 R0															
PERIOD COVERED October 1, 1979 to September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Response of mammalian cells exposed to chemotherapeutic drugs and continuous radiation.																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">James B. Mitchell, Ph.D.</td> <td style="width: 20%;">Radiation Biologist</td> <td style="width: 10%;">ROB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>Nirmolini Soares, M.S.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Thomas Bryant, B.S.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI:	James B. Mitchell, Ph.D.	Radiation Biologist	ROB	NCI	Other:	Nirmolini Soares, M.S.	Biologist	ROB	NCI		Thomas Bryant, B.S.	Biologist	ROB	NCI
PI:	James B. Mitchell, Ph.D.	Radiation Biologist	ROB	NCI													
Other:	Nirmolini Soares, M.S.	Biologist	ROB	NCI													
	Thomas Bryant, B.S.	Biologist	ROB	NCI													
COOPERATING UNITS (if any) None																	
LAB/BRANCH ROB																	
SECTION Radiobiology																	
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205																	
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0															
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) Cell killing and cell cycle kinetics will be studied for combinations of various chemotherapeutic drugs and continuous low dose rate radiation (5-300 rad/h). In addition, the morphological changes of cells exposed to these combinations of treatment will be documented by time lapse photography.																	

Project Description:

Objectives: The objectives of this project are to determine if there are combinations of continuous low dose rate radiation and chemotherapeutic drugs that will provide more cell killing to tumor cells (in vitro tumor cell lines) than to normal tissue cell lines.

Methods Employed: In vitro cell cultures will be exposed to the various agents mentioned above and assayed for cellular reproductive integrity using conventional tissue culture techniques.

Major Findings: The project is in the early stages of development. Experiments will begin when a Cobalt-60 irradiator is finally acquired and installed.

Significance to the Research Program of the Institute: These studies should provide a better understanding of interactions between radiation and drugs, which might be of value to clinical radiotherapy.

Proposed Course: Finish organizing in vitro laboratories, install Cobalt-60 unit, and initiate preliminary experiments.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM U6321-01 R0												
PERIOD COVERED October 1, 1979 to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Dose-rate effects on aerated and hypoxic cells grown in culture.														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: James B. Mitchell, PH.D.</td> <td style="width: 30%;">Radiation Biologist</td> <td style="width: 10%;">ROB</td> <td style="width: 20%;">NCI</td> </tr> <tr> <td>Other: Nirmolini Soares, M.S.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td>Thomas Bryant, B.S.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI: James B. Mitchell, PH.D.	Radiation Biologist	ROB	NCI	Other: Nirmolini Soares, M.S.	Biologist	ROB	NCI	Thomas Bryant, B.S.	Biologist	ROB	NCI
PI: James B. Mitchell, PH.D.	Radiation Biologist	ROB	NCI											
Other: Nirmolini Soares, M.S.	Biologist	ROB	NCI											
Thomas Bryant, B.S.	Biologist	ROB	NCI											
COOPERATING UNITS (if any) None														
LAB/BRANCH Radiation Oncology Branch														
SECTION Radiobiology Section														
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205														
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES XX ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study the effects of <u>ionizing radiation</u> with respect to <u>cell killing</u> and <u>cell cycle</u> perturbations to cells grown in culture. Particular <u>emphasis</u> will be placed on studying the response of cells to varying dose rates of radiation under both aerated and hypoxic culture conditions. Both continuous and fractionated irradiation schedules will be studied.														

Project Description:

Objectives: The objective of the proposed project is to obtain a better understanding of the nature of lesions and processes leading to cell reproductive death and to study the interrelationships of factors which influence radiosensitivity, with an emphasis on their implications for clinical radiotherapy.

Methods Employed: In vitro cell reproductive integrity will be assayed by the single cell plating techniques for attached cells. Cells will be exposed to various dose rates of radiation either under aerated or hypoxic conditions. Oxygen enhancement ratios (OER) will be determined.

Major Findings: The project is in the early stages of development. Experiments will begin when a Cobalt-60 irradiation is finally acquired and installed.

Significance to the Research Program of the Institute: These studies should provide a better understanding of the effects of dose rate/fractionation on the OER. Since there is a good deal of information that indicates that hypoxic cells in tumors represent a problem for radiotherapy, these studies could lead to more efficient methods of sterilizing hypoxic cells.

Proposed Course: Finish organizing in vitro laboratories, install Cobalt-60 unit, and initiate preliminary experiments.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06322-01 R0
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) The response of human hematopoietic stem cells (CFUc) exposed to radiation and hyperthermia.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Richard Bromer, M.D. James B. Mitchell, Ph.D. Other: Nirmolini Soares, M.S. Thomas Padikal, Ph.D.	Clinical Associate Radiation Biologist Biologist Physicist	ROB NCI ROB NCI ROB NCI ROB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Radiation Oncology Branch		
SECTION Radiobiology		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES XX ☐ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline keywords)		
By using a soft agar in vitro plating technique, the survival of human hematopoietic stem cells (CFUc) exposed to radiation and hyperthermia alone and in various combinations will be studied. These studies should provide important information on the response of a normal human tissue to these treatment modalities.		

Project Description:

Objectives: The objective of this project will be to determine the extent of cell killing that results from treatment of CFUc's with hyperthermia and radiation.

Methods Employed: Bone marrow cells from normal donors will be plated in soft agar dishes and treated with various schedules of hyperthermia/radiation.

Major Findings: Results are too preliminary to report on.

Significance to the Research Program of the Institute: Data of this nature may be useful in predicting bone marrow toxicity in patients being treated by combination of these modalities.

Proposed Course: Continue to accrue patients.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06323-01 R0
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Radiotherapeutic effects on hematopoietic stem cells in man.		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	Richard Bromer, M.D. Ross Abrams, M.D. James Mitchell, Ph.D. Allen Lichter, M.D. James Schwade, M.D. Joel Tepper, M.D. Eli Glatstein, M.D.	Clinical Associate Clinical Associate Radiation Biologist Radiotherapist " " " "
		ROB NCI ROB NCI ROB NCI ROB NCI ROB NCI ROB NCI ROB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Radiation Oncology Branch		
SECTION Radiobiology		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER XX ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
To investigate, in man, the systemic hematopoietic toxicity induced by regional radiotherapy during the management of neoplastic disease. Special emphasis will be placed on identifying, in both the peripheral blood and bone marrow, the quantitative, morphologic, and functional correlates of this toxicity using presently available in vitro methodology for studying hematopoietic and stem cell activity. Studies will be made both acutely and during periods of hematopoietic recovery.		

Project Description:

Objectives: (1) To determine the effects of regional radiotherapy on the unirradiated bone marrow.

(2) To investigate mechanisms of bone marrow repopulation after depletion caused by ionizing irradiation.

(3) To determine the effects of ionizing irradiation on peripheral blood bone marrow CFUc and CFUc cell cycle kinetics.

Methods Employed: Patients undergoing regional radiotherapy will have serial quantitative CFUc assays done from unirradiated bone marrow.

Major Findings: The study has only recruited 3 patients to date and the data is too premature to analyze.

Significance to the Research Program of the Institute: Observations in animals suggest that during regional radiotherapy, unirradiated bone marrow sites may be compromised in terms of proliferative and regenerative capacity. If it exists in man, this toxicity should be further defined and characterized in order to plan optimally the sequencing and design of treatment regimens incorporating multiple radiotherapy ports and combined modality regimens incorporating both radiotherapy and chemotherapy.

Proposed Course: Continue to accrue patients.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06324-01 R0																				
PERIOD COVERED October 1, 1979 to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Protection of Radiation Injury in Normal Tissues																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Elizabeth L. Travis, Ph.D.</td> <td style="width: 20%;">Cancer Expert</td> <td style="width: 10%;">ROB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>John F. Fowler, Ph.D., D.Sc.</td> <td>Visiting Scientist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Anne Marie DeLuca, B.Sc.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Charles J. Sanders</td> <td></td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI:	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI	Other:	John F. Fowler, Ph.D., D.Sc.	Visiting Scientist	ROB	NCI		Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI		Charles J. Sanders		ROB	NCI
PI:	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI																		
Other:	John F. Fowler, Ph.D., D.Sc.	Visiting Scientist	ROB	NCI																		
	Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI																		
	Charles J. Sanders		ROB	NCI																		
COOPERATING UNITS (if any) None																						
LAB/BRANCH Radiation Oncology Branch																						
SECTION Radiobiology Section																						
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205																						
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0																				
CHECK APPROPRIATE BOX(ES)																						
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER																						
☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords)																						
A series of sulfhydryl compounds have been developed by Walter Reed Army Institute, which protect normal tissues against radiation injury. WR 2721, one drug in this series gives a dose modifying factor (i.e., ratio of radiation doses that give the same level of biological damage with the drug as without the drug) ranging from 1.2 to 2.5 depending on the normal tissue tested. This drug has been shown not to protect murine tumors from radiation. This drug is now being used in clinical trials in radiation therapy. The purpose of this work is to study the ability of this drug to protect a rapidly proliferating and slowly proliferating tissue, i.e., skin and lung respectively, in mice, and to compare this drug with other radioprotectors available from Walter Reed.																						

Project Description:

Objectives: To assess the ability of sulphhydryl compounds to protect against radiation injury in skin and lung of mice to determine the time of protection after various routes of administration, to determine if any of the drugs protect tumors.

Methods Employed:

Skin: One foot of a mouse is irradiated in a specially constructed irradiation jig. No anaesthetics are used for the procedure. Damage is assayed by quantifying the erythema, alopecia, dry and wet desquamation.

Lung: Both lungs of a mouse are irradiated in a specially constructed jig in which the rest of the body is shielded. No anaesthetic is used for the irradiation. Lung damage is assayed using a plethysmographic technique. (Travis et al., Brit. J. Radiol.)

Major Findings: This project has begun only recently and little data is available. However, one experiment in which WR 2721 was administered IP at times before irradiation ranging from 5 mins. to 1 hr. indicates that optimum protection occurred when the drug was given 30 min. before irradiation. Data from an IV experiment is not presently available.

Significance to the Research Program of the Institute: Radioprotectors may offer a means of improving the therapeutic ratio in radiotherapy.

Proposed Course: To be continued.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06325-01 R0																
PERIOD COVERED October 1, 1979 to September 30, 1980																		
TITLE OF PROJECT (80 characters or less) In vivo radiobiology.																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: Elizabeth L. Travis, Ph.D.</td> <td style="width: 30%;">Cancer Expert</td> <td style="width: 10%;">ROB</td> <td style="width: 20%;">NCI</td> </tr> <tr> <td>Others: John F. Fowler, Ph.D., D.Sc.</td> <td>Visiting Scientist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td>Anne Marie DeLuca, B.Sc.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td>Charles J. Sanders</td> <td></td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI: Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI	Others: John F. Fowler, Ph.D., D.Sc.	Visiting Scientist	ROB	NCI	Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI	Charles J. Sanders		ROB	NCI
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Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI															
Charles J. Sanders		ROB	NCI															
COOPERATING UNITS (if any) None																		
LAB/BRANCH Radiation Oncology Branch																		
SECTION Radiobiology																		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, MD 20205																		
TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 5.0	OTHER: 																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords)																		
The purpose of the project is to establish an <u>in vivo</u> radiobiology research program. The major efforts of the research projects are directed towards radiobiology as it applies to clinical radiotherapy and towards basic radiobiology. The major emphasis of this program is on normal tissue injury after radiation and/or drugs.																		

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06326-01 RO																				
PERIOD COVERED October 1, 1979 to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Early and late radiation damage in mouse lung.																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
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COOPERATING UNITS (if any) None																						
LAB/BRANCH Radiation Oncology Branch																						
SECTION Radiobiology																						
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☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords)																						
Radiation causes two distinct histological changes in the mouse lung which occur at clearly separated times; radiation pneumonitis at 4-5 months after treatment (early damage) and fibrosis at longer than 9 months post treatment (late damage). It is possible to dissociate these 2 types of damage, i.e., cause little early reaction while inducing a severe late reaction. This suggests that 2 different cell types might be responsible for the 2 types of damage. It is the purpose of this study to study the conditions under which early and late damage can be dissociated, to define the radiobiological characteristics of the 2 types of damage, and define those cells which are involved in each type of damage. Answers to these questions may enable us to reduce late damage in the lung.																						

Project Description:

Objections: To define the conditions under which early and late damage in the lung can be dissociated; to define the radiobiological characteristics of early and late damage, to define the cells involved in each type of damage.

Methods Employed: The whole thorax of mice is irradiated with X-rays using a specially constructed jig which shields the rest of the body. Thus, only the lung injury is expressed. The procedure requires no anaesthetic. Fractionated doses of X-rays and low dose late radiation will be used. Early and late lung damage is assayed by changes in breathing rate. Histological changes are scored and autoradiography will be used to look for proliferation of cells in the lung.

Major Findings: This project has begun only recently; therefore, no data is available.

Significance to the Program of the Institute: Normal tissues are the dose limiting factor in radiotherapy, late fibrosis in the lung being a critical reaction. It is important that those conditions which can result in severe late fibrosis in the lung be defined and that conditions in which early reactions do not predict for late fibrosis be identified. Identification of the cell types involved in late damage may enable us to reduce this type of damage.

Proposed Course: To be continued.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06327-01 R0																				
PERIOD COVERED October 1, 1979 to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) Normal tissue protection from drug induced injury.																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Elizabeth L. Travis, Ph.D.</td> <td style="width: 25%;">Cancer Expert</td> <td style="width: 10%;">ROB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>John F. Fowler, Ph.D., D.Sc.</td> <td>Visiting Scientist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Anne Marie DeLuca, B.Sc.</td> <td>Biologist</td> <td>ROB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Charles J. Sanders</td> <td></td> <td>ROB</td> <td>NCI</td> </tr> </table>			PI:	Elizabeth L. Travis, Ph.D.	Cancer Expert	ROB	NCI	Other:	John F. Fowler, Ph.D., D.Sc.	Visiting Scientist	ROB	NCI		Anne Marie DeLuca, B.Sc.	Biologist	ROB	NCI		Charles J. Sanders		ROB	NCI
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INSTITUTE AND LOCATION NIH, NCI, Bethesda, MD 20205																						
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5																				
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SUMMARY OF WORK (200 words or less - underline keywords) Sulfhydryl compounds, a class of radioprotectors, were originally developed to protect against nitrogen mustard gas. Therefore, those compounds may be useful in protecting against chemotherapeutic drugs whose action is mainly alkylation. The purpose of this work is to study the ability of WR 2721 and other radioprotectors to protect critical normal tissues against damage from chemotherapeutic agents.																						

Project Description:

Objectives: To determine the ability of WR 2721 to reduce injury in the lungs and teeth of mice after administration of cyclophosphamide.

Methods Employed: Groups of mice will be given the protective drug at various times before administration of a range of cyclophosphamide doses. Other groups of mice will be given cyclophosphamide alone. Lung damage will be assayed in both groups of mice by measuring breathing rate. A system for scoring tooth deformities is being developed.

Major Findings: The study has just begun and no data is available.

Significance to the Research Program of the Institute: Normal tissue toxicity is a limiting factor in the treatment of malignant diseases with chemotherapeutic agents. If radioprotectors reduce the damage to critical normal tissues such as lung, bone marrow, and gut, then the therapeutic ratio will be increased, i.e., there will be less morbidity.

Proposed Course: To be continued. A range of radioprotectors will be tested against a range of chemotherapeutic drugs. Various routes of administration will be tested for both drugs to determine the route which gives the best protection.

Publications: None.

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1979 to September 30, 1980

Clinical efforts in the Surgery Branch continue to emphasize combined modality approaches to the treatment of cancer. Prospective randomized protocols in the treatment of soft-tissue sarcomas are exploring the role of adjuvant chemotherapy as well as the role of limb-sparing surgery. Active clinical protocols are in progress evaluating the effect of adjuvant chemo- and immunotherapy in the treatment of melanoma and colo-rectal cancers. New programs in the treatment of testicular cancer, esophageal cancer have been initiated as well as exploratory attempts to evaluate the role of intraoperative radiation therapy in the treatment of cancer. Active laboratory research programs in tumor immunology and in tumor metabolism are also in progress.

Surgical procedures on consultations performed in the Surgery Branch from April 1, 1979 to March 31, 1980, are presented in Tables I to IV.

TABLE I
Cases - General Surgery
April 1, 1979 - March 31, 1980

<u>General:</u>	Abdominal-perineal resection	2
	Aortocaval lymph node dissection and biopsy for ovarian cancer staging	14
	Colon/bowel resection	30
	Cholecystectomy	7
	Colonoscopy	25
	Electrofulguration	1
	Exploratory laparotomy	36
	Gastrectomy	1
	Gastroscoy	2
	Gastrotomy	2
	Hepatic resection	3
	Herniorrhaphy/hernia repair	7
	Laparoscopy	3
	Major soft tissue or muscle group excision	15
	Pancreatectomy - partial/total	10
	Peritoneoscopy	4
	Revision of colostomy	4
	Radiation therapy - intraoperative	5
	Splenectomy	4
	Staging laparotomy + splenectomy for lymphoma	31
	Wound exploration/secondary closure	7
<u>Surgery for</u>		
<u>Melanoma:</u>	Wide excision with/without STSG	18
	Wide excision + axillary LND	10
	+ inguinal LND/superficial groin	5
	+ radical neck LND	3
	Lymph node dissection: Axillary	20
	Superficial inguinal	9
	Deep inguinal	2
	Radical neck	2
	Eptitrochlear	1
	Excision solitary nodules/nevi	14
<u>Head & Neck:</u>	Radical neck + other resection	5
	Tracheotomy	13
	Parotidectomy	1
	Other major cases	4
<u>Plastic:</u>	Wound debridement - major	4
	Skin grafting	6
	Scar revision	1

TABLE I (continued)

<u>GYN:</u>	Hysterectomy	1
	Salpingo-oophorectomy	1
	D&C	1
<u>GU:</u>	Aortocaval/retroperitoneal lymph node dissection	19
	Cystoscopy + other procedures, biopsy	79
	Cystotomy, suprapubic	3
	Debulking procedure	13
	Epidostomy	1
	Looposcopy	3
	Loop revision	1
	Marshall-Marchetti	2
	Orchiectomy/orchiopexy	4
	Nephrostomy/nephrolithotomy/nephrectomy	9
	Prostatic biopsy	4
	Repair varicocele	1
	Superficial groin dissection	1
	Transureteroureterostomy/ureterolithotomy	2
	Transureteroprostatectomy	9
	Testicular biopsy	8
<u>Breast:</u>	Breast biopsy	76
	Simple mastectomy	3
	Modified radical mastectomy	29
	Axillary node dissection with biopsy	11
<u>Orthopedics:</u>	Below knee amputation	4
	Above knee amputation	10
	Hip disarticulation	5
	Hemipelvectomy	10
	Forearm amputation	2
	Forequarter amputation	1
	Ray amputation	1
	Claviclectomy	1
	Fibulectomy	1
	Amputation toes	2
	Muscle biopsy	2
	Stump revision	7
	Bone biopsy	22

TABLE I (continued)

<u>Endocrine:</u>	Parathyroidectomy	38
	Parathyroid implant/removal	3
	Adrenalectomy	7
	Thyroidectomy - complete/subtotal	20
	Radical neck LND - met. thyroid ca.	1
	Mediastinal exploration	2
	Enucleation insulinoma	1
	Excision pheochromocytoma	1
<u>Vascular:</u>	A-V shunt/shunt revision/shunt removal	37
	Broviac/Hickman catheter placement	10
	Subclavian CVP line placement	10
	Endarterectomy/embolectomy	7
	Arterial/venous bypass	4
	Ligation IVC	1
	Profundoplasty	1
	Resection aneurysm	1
<u>Thoracic:</u>	Unilateral thoracotomy	
	Biopsy nodules	47
	Biopsy nodules, chest wall resection	1
	Lobectomy	16
	Pneumonectomy	2
	Decortication	1
	Drainage, empyema	3
	Bilateral thoracotomy/median sternotomy	19
	Resection mediastinal mass	3
	Bronchoscopy	38
	Mediastinoscopy	1
	Esophagoscopy	17
	Esophagogastrectomy	7
	Rib biopsy	3
<u>Minor:</u>	Incision and drainage of abscess	21
	Biopsy: Node	64
	Tissue mass, NOS	68
	Insertion/removal abdominal catheter for chemotherapy	32
	Wound repacking	3
	Excision toenail	2

TABLE II
Consultants - Surgery
April 1, 1979 - March 31, 1980

<u>Plastic Surgery:</u>	Biopsy mass	2
	Epidermectomy	1
	Major skin grafting	4
	Mammoplasty	
	Augmentation with mastectomy	8
	Reduction	1
	Myocutaneous skin flap transfer	1
<u>Vascular:</u>	Placement vena cava umbrella	1
	Aorto-carotid bypass	1
	Renal artery bypass	1
<u>Thoracic:</u>	Bronchoscopy	8
<u>Neurosurgical:</u>	Excision tumor lumbar spine	1
<u>Gynecological:</u>	D&C	1
	Exploratory laparotomy	2
	Laparoscopy	5
	Total abdominal hysterectomy	1
	Tubal ligation	2
<u>Orthopedic:</u>	Bone biopsy	4
	Casting leg	1
	Manipulation, knee	1
	Muscle release	1
<u>ENT:</u>	Creation nasal antral window	4
	Laryngoscopy/pharyngoscopy/esophagoscopy	8
	Myringotomy, bilateral	5
	Polypectomy	3
	Tonsillectomy	2
	Tracheostomy	1
	Tympanoplasty	2
<u>Dental:</u>	Intraoral biopsies	1

TABLE III
Individual Written Intramural Consultations Received by Surgery Branch, NCI
Listed by Institutes*
April 1, 1979 - March 31, 1980

NCI	155
NIAMDD	47
NHLBI	35
NIAID	73
NIMH	20
NINCDS	42
NIDR	3
NICHHD	7
NEI	3
OTHER	<u>43</u>
TOTAL	428

*This tabulation includes only formal written consultations submitted to the Surgery Branch office. Many additional consultations are received by Surgery Branch physicians directly by telephone or personal contact. These are not included in this tabulation.

TABLE IV
Annual Statistical Report
Surgical Services Department
April 1, 1979 - March 31, 1980

Patient	NCI	NHLBI	NINCDS Neuro. Muscle	NIAMDD	NIAID	NIDR	NIMH	NICHHD	NEI	Consult- ants	Total # Pts. Operated
NCI	Major	759	1	8	-	2	-	-	-	45	815
	Minor	214.5	1.5	-	1	-	-	-	1	8	226
NHLBI	Major	30	295	-	-	-	-	-	-	-	1041
	Minor	15	19	-	2	2	-	-	4	-	333
NINCDS	Major	11	10	62	15	3	-	-	-	-	367
	Minor	13	-	5	216	-	-	-	-	-	102
NIAMDD	Major	101	-	3	-	-	-	1	-	-	234
	Minor	23	-	1	1	-	-	-	-	8	115
NIAID	Major	58	-	2	-	-	-	-	-	5	30
	Minor	15	-	4	-	-	-	-	-	8	73
NIDR	Major	-	-	-	-	-	4	-	-	7	26
	Minor	-	-	-	-	-	-	-	-	-	4
NIMH	Major	9	-	-	-	-	-	-	-	3	3
	Minor	5	-	-	-	-	-	-	-	-	9
NICHHD	Major	11	-	4	-	-	-	4	-	-	5
	Minor	4	-	-	1	-	-	-	-	2	21
NEI	Major	-	-	1	-	-	-	-	-	-	5
	Minor	-	-	5	-	-	-	-	22	1	24
MAJOR TOTAL		979.0	306.0	82	18	9	0	6	0	5	22
MINOR TOTAL		289.5	20.5	5	228	1	0	0	0	4	23
GRAND TOTAL		1268.5	326.5	87	246	10	0	6	0	5	27
										92*	2068**

*Includes 38 anesthesia procedures.

**Of these 2068 surgical procedures, 182 patients were on O.P.D. status and 41 procedures were performed on the nursing units. There were 171 emergency procedures.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 CM 03800-10 SURG
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Surgical Consultations and Collaborative Research Involving Surgical Services at the National Institutes of Health		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	S. A. Rosenberg and entire staff of G. D. Aurbach J. L. Doppman E. Glatstein J. Robbins J. Costa R. C. Young A. S. Levine J. Gardner	Chief of Surgery, NCI Surgery Branch, NCI Chief, Metabolic Dis. Br. Chief, Diag. Radiol. Dept. Chief, Rad. Oncol. Br. Chief, Clin. Endocrin. Br. Lab. of Pathology Chief, Medicine Branch Chief, Ped. Oncol. Br. Chief, Digestive Dis. Br.
		SURG NCI SURG NCI MD NIAMDD DR CC RO NCI CE NIAMDD LP NCI M NCI PO NCI DD NIAMDD
COOPERATING UNITS (if any)		
LAB/BRANCH Surgery Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 6.0	PROFESSIONAL: 4.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The Surgery Branch of the National Cancer Institute are the general surgeons and general <u>surgical consultants</u> to the entire National Institutes of Health. In this role we see patients for elective consultations as well as all emergency general surgical problems. Many collaborations on clinical studies have resulted from these consultative efforts.		

Introduction

Investigators in the Surgery Branch of the National Cancer Institute are the general surgeons and general surgical consultants to the entire National Institutes of Health. In this role we see patients in primarily two capacities. Firstly, we see patients in consultation for all general surgical and specialty surgical problems except for the specialties of cardiac and orthopedic surgery. The Surgery Branch answers all emergency as well as elective surgical consultations and provides 24 hour coverage for surgical emergencies that may arise in the Clinical Center Hospital.

Secondly, the Surgery Branch collaborates in the procurement of tissues for studies required by other investigative units. The degree of involvement of the Surgery Branch in the planning and execution of these studies is variable. The Surgery Branch often plays an instrumental role in the design of these studies while in other collaborations, the Surgical Service merely provides tissues.

Approximately 40% of the clinical surgical effort of the Surgery Branch is devoted to these consultative and collaborative studies.

A complete listing of surgical procedures performed by the Surgery Branch is presented in Table I. Surgery performed by surgical consultants operating within the Surgery Branch is listed in Table II.

A list of consultations received from other NCI Branches as well as other NIH Institutes is shown in Table III.

Project Description: Selected projects are presented below to provide examples of the nature of Surgery Branch collaborative efforts.

- | | |
|--------------------|--------------------------------------|
| <u>Part I.</u> | <u>Endocrine Surgery</u> |
| <u>Part II.</u> | <u>Thoracic and Vascular Surgery</u> |
| <u>Part III.</u> | <u>Nutritional Support</u> |
| <u>Part IV.</u> | <u>Ovarian Cancer</u> |
| <u>Part V.</u> | <u>Tenckhoff Catheters</u> |
| <u>Part VI.</u> | <u>Vascular Access</u> |
|
<u>Part I.</u> |
<u>Endocrine Surgery</u> |

All the endocrine surgery for the Clinical Center is provided by the Surgical Metabolism Section of the Surgery Branch.

In the period 4/1/79-4/1/80, 40 patients were operated on for hyperparathyroidism. Twenty-eight of the 40 were undergoing a reexploration following a failed previous exploration.

A vigorous program of preoperative localization, intraoperative localization, assessment of adequacy of surgical resection, and human cryopreserved autografting is being vigorously pursued both clinically and in the laboratory.

The remaining endocrine tumors tend to be dominated by the islet cell tumors of the pancreas, with adrenal tumors being less common but active surgical involvement in the management and treatment of pheochromocytoma, adrenal Cushing's disease, and hyperaldosteronism has continued.

Part II. Thoracic and Vascular Surgery

Consultative services for thoracic and vascular surgical problems are handled through the Surgery Branch. Eleven major and 20 minor thoracic surgical procedures have been performed since July 1979. Likewise 11 major vascular procedures, including carotid endarterectomies, renal artery bypass, and aorto-carotid bypass operations have been performed along with 6 minor cases.

Part III. Nutritional Support

The Surgery Branch continues to mount a major effort in supporting patients nutritionally by intravenous feeding throughout the National Institutes of Health. The Surgery Branch has been responsible for 1236 days of total parenteral nutrition between the period of 2/1/79 and 2/1/80.

Part IV. Ovarian Cancer

Studies of ovarian carcinoma are undertaken in Medicine Branch protocols with the cooperation of the Surgery Branch. Adjuvant systemic melphalan chemotherapy is being compared with intraperitoneal radioactive phosphorus for high-risk patients following complete surgical tumor resections. Intraperitoneal chemotherapy is evaluated both as an adjuvant in early-stage patients and as definitive therapy in certain patients with advanced disease. Patients with incomplete surgical tumor resections or with disseminated disease are treated with various combinations of systemic chemotherapy. The Surgery Branch collaborates with the Medicine Branch in ovarian cancer studies by providing surgical evaluations and services, as well as performing definitive resections, staging laparotomies, explorations for complications or failures of treatment, and peritoneal catheter placements. During 1979, the Surgery Branch performed operative procedures on 37 patients in ovarian cancer protocols.

Part V. Tenckhoff Catheters

Tenckhoff catheters have been used for several years for peritoneal dialysis in patients with chronic renal failure. Using these catheters, direct administration of chemotherapeutic agents is possible into the peritoneal cavity. The conduct of these studies has been under the general direction of Dr. Charles Meyers of the Medical Pharmacology Branch. Phase I and Phase II trials of intraperitoneal 5-FU have been completed for ovarian cancer. An adjuvant 5-FU trial for lymph node positive colon and rectal cancer patients is currently in progress. Phase I trials with intraperitoneal Adriamycin have begun. The Surgery Branch is responsible for the insertion and removal of Tenckhoff catheters on protocol patients. Thirty-eight catheters were inserted 4/1/79-3/31/80.

Part VI. Vascular Access

The Surgery Branch accepts consults from the Clinical Center to provide means of vascular access. Renal dialysis, plasmaphoresis, drug and blood products infusion and blood withdrawal are some of the indications for vascular access procedures in selected patients. Since July 1979, 12 arterio-venous fistulae have been constructed, 5 Scribner shunts inserted, and 5 Hickman/Broviac silastic catheters placed.

Publications:

Auda, S.P., Brennan, M.F. and Gili, J.R.: Evolution of the surgical management of primary aldosteronism. *Ann. Surg.* 191:1-7, 1980.

Austin, E.H. and Flye, M.W.: The treatment of recurrent malignant pleural effusion. *The Ann. Thorac. Surg.* 28:190-203, 1979.

Baskies, A.M., Chretien, P.B., Wolf, G.T., and Weiss, J.F.: Correlation of serum immune-reactive proteins with clinical tumor stage in patients with squamous carcinoma of the head and neck and nasopharyngeal carcinoma. *Surg. Forum*, Vol. XXX, 516-518, 1979.

Baskies, A.M., Chretien, P.B., Yang, C., Wolf, G.T., et al.: Serum glycoproteins and immunoglobulins in nasopharyngeal carcinoma. *Am. J. Surg.* 133:478-488, 1979.

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PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Clinical Studies in Cancer Surgery

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Rosenberg	Chief of Surgery, NCI	SURG	NCI
	and entire staff of	Surgery Branch, NCI	SURG	NCI
Other:	J. Costa	Lab. of Pathology	LP	NCI
	W. Terry	Chief, Immunology Br.	I	NCI
	R. C. Young	Chief, Medicine Branch	M	NCI
	B. A. Chabner	Head, Biochem. Pharm. Sect.	LCHPH	NCI
	M. Lippman	Senior Investigator	M	NCI
	E. Glatstein	Chief, Rad. Oncol. Br.	RO	NCI
	J. L. Doppman	Chief, Diag. Radiol. Dept.	DR	CC
	A. Levine	Chief, Pediatric Oncol. Br.	PO	NCI

COOPERATING UNITS (if any)

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

10.6

PROFESSIONAL:

7.6

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less, underline keywords)

The Surgery Branch has a variety of studies investigating innovative therapies for patients with malignant disease. The major emphasis of these studies is in the treatment of malignant melanoma, soft tissue sarcomas, osteogenic sarcomas, and colorectal cancer. The major emphasis in Surgery Branch cancer therapy is in adjunctive therapy with emphasis on the use of multiple treatment modalities in addition to surgery.

Project Description:

<u>Part I.</u>	<u>Malignant Melanoma</u>
<u>Part II.</u>	<u>Soft Tissue Sarcomas</u>
<u>Part III.</u>	<u>Osteogenic Sarcoma</u>
<u>Part IV.</u>	<u>Testicular Cancer</u>
<u>Part V.</u>	<u>Colorectal Cancer</u>
<u>Part VI.</u>	<u>Total Parenteral Nutrition</u>
<u>Part VII.</u>	<u>Breast Cancer</u>
<u>Part VIII.</u>	<u>Quality of Life Studies</u>
<u>Part IX.</u>	<u>Endoscopy</u>
<u>Part X.</u>	<u>Computer Applications</u>
<u>Part XI.</u>	<u>Esophageal Cancer</u>
<u>Part XII.</u>	<u>Intraoperative Radiotherapy</u>
<u>Part XIII.</u>	<u>Resection of Pulmonary Metastases</u>
 <u>Part I.</u>	 <u>Malignant Melanoma</u>

The Surgery Branch is involved in several studies designed to improve the treatment of patients with malignant melanoma. A prospective randomized study of the intralesional injection of BCG into primary cutaneous melanoma lesions was begun in 1975. Twenty-six patients have been randomized in this study. A statistically significant improvement in disease-free survival ($p < .05$) is present in the patients treated with intralesional BCG compared to patients receiving standard surgical treatment alone. For the treatment of multiple cutaneous recurrences of melanoma, the Surgery Branch is investigating the use of BCG cell walls on oil droplets as an alternative to the use of viable BCG for the treatment of these lesions. In conjunction with the Immunology and Medical Oncology Branches, the Surgery Branch is conducting a prospective randomized trial of 3 adjuvant treatments in addition to surgery for the treatment of patients with Stage II melanoma. Approximately 180 patients have been randomized into these protocols. Preliminary analysis has revealed no significant differences among any of the treatment groups.

Part II. Soft Tissue Sarcomas

The Surgery Branch has completed evaluation of 55 patients treated with adjuvant chemotherapy following surgery for the treatment of soft tissue sarcomas. Dramatic improvements in disease-free interval and survival have been obtained by the use of chemotherapy, although some complications of the chemotherapy have been seen. A prospective randomized protocol is now in progress evaluating the efficacy of adjuvant chemotherapy. To the present, 60 patients have been randomized into this study. It is too early to evaluate results. The Surgery Branch is also conducting a prospective randomized trial comparing standard amputation with conservative excision and radiation therapy in the local control in patients with soft tissue sarcomas. Although it is too early for definitive analysis, it appears as if radiation therapy is effective in improving local control in this disease.

Part III. Osteogenic Sarcoma

Fifty-five patients have been entered into a study evaluating use of high-dose methotrexate for the adjuvant treatment of patients with osteosarcomas. Continuous disease-free interval has been improved from 20% in historical controls to 38% in adjuvant chemotherapy treated patients. The aggressive use of thoracotomy in these patients has led to a dramatic increase in disease-free survival. Currently 70% of patients are alive and NED following diagnosis of osteogenic sarcoma. This represents a dramatic improvement over survival figures prior to the onset of this protocol.

A new protocol has been initiated comparing, in a prospective randomized fashion, adjuvant therapy with high-dose methotrexate compared to surgery alone in patients with osteogenic sarcoma. Ten patients have been entered into this study.

Part IV. Testicular Cancer

The main objective of this protocol is to assess the role of cytoreductive surgery combined with intensive chemotherapy in bulky Stage III testicular cancer. After extensive staging procedures, Stage III bulky tumors are randomized to chemotherapy alone or chemotherapy with cytoreductive surgery. In the surgery and chemotherapy arm, 8 of 18 patients have died and the remaining 10 patients are in remission. In the chemotherapy arm, 8 patients are dead and 9 in remission; of the 9 patients in remission, 4 had subsequent debulking surgery.

Part V. Colorectal Cancer

At the present time three protocols for the study of patients with colorectal cancer are operative. The first protocol concerns the postoperative management of patients who have had lymph node positive colon or rectal cancer removed. These patients are carefully followed with monthly CEA assays, 3-monthly abdominal ultrasound and CAT scans, and monthly physical examination. By so doing we wish to pick up early recurrences for "second look surgery" as well as determine optimal follow-up regimens. In the second protocol the same group of patients are randomized to receive intravenous or intraperitoneal 5-FU. Ten patients have been entered into these studies. In the second study patients with local or regional colon or rectal recurrence undergo second look surgery randomized to receive or not receive radiation therapy.

Part VI. Total Parenteral Nutrition

The Surgery Branch is involved in three prospective randomized studies of the effect of total parenteral nutrition in the management of malignancy. The first, the diffuse histiocytic lymphoma protocol, has been concluded and shows that total parenteral nutrition given in a random fashion regardless of preceding weight loss is not able to improve the tolerance to an aggressive chemotherapeutic regimen.

The use of total parenteral nutrition in ablative chemotherapy for recurrent metastatic soft tissue sarcomas continues its slow accrual with the Pediatric Oncology Service.

The newly instigated esophageal protocol which involves an aggressive radiation with and without misonidazole preoperatively for carcinoma of the esophagus has included an intravenous nutritional support arm which is randomly allocated. So far 13 patients have been entered into this protocol and 5 have completed their therapy.

Part VII. Breast Cancer

Under protocol 79-C-111 which began accrual of patients during the summer of 1979, 19 patients have been entered through April 1980. Patients 70 years of age or younger with clinical Stage I or II unilateral breast cancer are randomly assigned to receive total mastectomy with axillary dissection, or excisional biopsy with axillary dissection followed by radiotherapy to the affected breast. Of the 19 patients, 10 have been randomized to the mastectomy group, and 9 to the radiotherapy group. With at least one positive axillary node, 8 patients have entered into adjuvant chemotherapy with IV Adriamycin and oral Cytosan. With a median follow-up of 4 months there has been one recurrence. Patients who receive mastectomy will be given an opportunity to have reconstruction. An increased rate of accrual is hoped for during the next year.

Part VIII. Quality of Life Studies

Although survival statistics in the various arms of a controlled clinical trial are not difficult to obtain, the change that various treatment regimens cause in the quality of life is more difficult to quantitate. Therefore, a Quality of Life Assessment (QOLA) has been constructed to measure the economic, sociologic, and psychologic cost of cancer treatments. This impact of therapy is considered to be valuable data useful to the physician who recommends a particular treatment plan to a patient. At present a retrospective study of patients undergoing therapy for soft tissue sarcoma is underway; a comparison of the quality of life change in patients undergoing a major amputation to those receiving limb-sparing surgery plus high-dose radiation therapy is being made. All patients studied to date received chemotherapy.

Part IX. Endoscopy

The Surgery Branch continues to collect clinical data to help define the appropriate role for laparoscopy in cancer diagnosis and treatment. Laparoscopic tubal ligation, as a consultative service, is available for appropriate patients. During the past year, 41 patients have undergone laparoscopy on the NCI surgical service. Surgery Branch colon and rectal cancer protocols promise to increase our utilization of this endoscopic procedure in the coming year.

Fiberoptic endoscopy of the lower GI tract is available as a consultative service function of the Surgery Branch. The utility of this procedure from both the diagnostic and therapeutic point of view of colon lesions is well established. Twenty-four colonoscopic procedures were performed in the past year. These included 14 diagnostic examinations and 10 snare polypectomies. Direct visual access to and ability to histologically sample tissues from the entire lower GI tract offer excellent opportunities for earlier diagnosis of colon malignancy. Premalignant lesions in patients with ulcerative colitis can be identified. Colonoscopy will play a role in the preoperative assessment of appropriate patients to undergo colon and rectal surgery.

Part X. Computer Applications

Data for Surgery Branch research continued to be collected and entered into 3 primary systems: (a) randomized protocols, (b) serum inventory and (c) nutrition studies. The basic objectives underlying the design of these systems are to: (1) ensure very high accuracy of the data and (2) permit information to be easily recorded, keyentered, verified, corrected, retrieved, and analyzed.

These systems are maintained on computers at the Division of Computer Resources and Technology. On the IBM-370 system, the text-editor WYLBUR is used for data entry, program maintenance, and remote job submission. The Time Sharing Option is used for graphic displays. On the DECsystem-10 computer, various software packages are used for entry of laboratory data and also for graphic displays. A Tektronix 4012 terminal is used for data retrieval and graphic display of

information. A high-speed Atlanthus T-1222 terminal is used for data entry and retrieval. An MFE-5000 terminal is used to enter data which has been recorded on cassette tape by an automatic analyzer

A simple measure of data processing activity is to report the current record counts for the various systems. The following counts show the status of the 3 systems as of May 1980:

<u>System</u>	<u>No. of Subjects</u>	<u>No. of Records</u>
(a) Randomized protocols	349	6,589
(b) Serum inventory	6,731	26,934
(c) Nutrition studies	371	31,792

In the serum inventory there are currently 42,994 vials of serum and 6,998 vials of lymphocytes collected in 20,203 drawings.

Data processing will continue to play a significant role in assisting Surgery Branch investigators to define and describe the characteristics of protocol populations.

Part XI. Esophageal Cancer

A study to determine the efficacy of a radiosensitizing drug, misonidazole, has been instituted in patients with esophageal carcinoma. In conjunction with the Radiation Oncology Branch, patients with squamous carcinoma of the mid or lower esophagus are radiated to 4000 rads with or without misonidazole. The patients then undergo esophageal resection with reconstruction by esophagogastrotomy. Six patients have completed the therapy since July 1979 and 3 more are under active treatment.

Part XII. Intraoperative Radiotherapy

The Surgery Branch has initiated investigations of the role of combined surgery and intraoperative radiation therapy for the local control of abdominal malignancies. A series of 5 pilot patients were treated in 1979. Patients underwent surgical resection of a variety of abdominal tumors, were transported under anesthesia from the operating room to the radiotherapy treatment facility, were treated with electron irradiation directly to the tumor bed through the open abdomen with vital structures shielded from the radiation beam, and were returned to the operating room for completion of surgery. No technical difficulties were encountered in the pilot series, and therefore investigative prospective randomized protocols were initiated in early 1980 to evaluate

combined surgical resection and intraoperative radiation in certain poor-prognosis malignancies. Protocols for the treatment of gastric and pancreatic cancers were established, comparing intraoperative radiotherapy with conventional external beam postoperative radiation in patients able to have all gross tumor surgically resected. Protocol for the treatment of retroperitoneal sarcomas was developed, comparing intraoperative and postoperative low-dose external beam radiation with conventional high-dose postoperative radiotherapy in patients with surgically resectable tumors; in addition, the use of adjuvant chemotherapy is evaluated in patients treated for retroperitoneal sarcomas. As of 5/1/80, 11 patients were evaluated for the intraoperative radiation protocols, with 6 entering study and undergoing randomizations.

Part XIII. Resection of Pulmonary Metastases

The Surgery Branch continues its protocol for the aggressive removal of metastatic sarcoma to the lung. After a thorough work-up to rule out metastatic disease at other sites, patients with locally controlled osteo or soft-tissue sarcoma are subjected to pulmonary resection. An attempt is made to remove all gross evidence of tumor while preserving as much pulmonary parenchyma as possible. Multiple procedures are often required. Patients with unilateral pulmonary metastases from melanoma are also considered for resection. These patients undergo formal mediastinal lymph node dissections in conjunction with the pulmonary resection. Since July 1979, 15 thoracotomies, 18 median sternotomies and 8 miscellaneous procedures have been performed.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03811-06-SURG
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) The Immunotherapy of Animal and Human Sarcomas		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	S. A. Rosenberg J. Strausser M. Lotze R. Almaraz T. Eberlein M. Rosenstein J. Brown I. Yron S. Schwarz H. Wexler P. Spiess C. Hyatt	Chief of Surgery, NCI Clinical Associate Clinical Associate Clinical Associate Clinical Associate Research Fellow Staff Fellow Visiting Fellow Bio. Lab. Tech. Biologist Biologist Biologist SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI
COOPERATING UNITS (if any)		
LAB/BRANCH Surgery Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 8.5	PROFESSIONAL: 5.0	OTHER: 3.5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Detailed studies of tumor-host immune interactions in animals and humans with sarcomas</u> are being performed in an attempt to develop new immunodiagnostic and immunotherapeutic techniques for diagnosis and treatment of these tumors. Immune response to murine sarcomas has been extensively evaluated and both tumor-specific and fetal antigens have been identified. Fetal antigens have been identified on human osteogenic sarcomas and these antigens are now being characterized. Attempts are being made to develop adoptive immunotherapeutic techniques utilizing transfer of cells from xenogeneic species to humans with cancer. Techniques for the prolonged growth of cytotoxic T cells have been developed, and attempts to use these cells for adoptive immunotherapy are in progress.		

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03812-06 SURG																												
PERIOD COVERED <u>October 1, 1979, to September 30, 1980</u>																														
TITLE OF PROJECT (80 characters or less) Studies with a Chemically-Induced Rat Colon Cancer Model																														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																														
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">A. R. Baker</td> <td style="width: 35%;">Senior Investigator</td> <td style="width: 15%;">SURG NCI</td> </tr> <tr> <td>Other:</td> <td>S. F. Stinson</td> <td>Pathologist</td> <td>TP NCI</td> </tr> <tr> <td></td> <td>R. M. Simon</td> <td>Chief, Biometric Res. Br.</td> <td>DCT NCI</td> </tr> <tr> <td></td> <td>N. P. Lang</td> <td>Visiting Scientist</td> <td>LID NCI</td> </tr> <tr> <td></td> <td>H. J. Rapp</td> <td>Chief, Lab. Immunobiol.</td> <td>LIB NCI</td> </tr> <tr> <td></td> <td>M. C. Chappell</td> <td>Bio. Lab. Tech. (Micro.)</td> <td>SURG NCI</td> </tr> <tr> <td></td> <td>R. McCauley</td> <td>Clinical Associate</td> <td>SURG NCI</td> </tr> </table>			PI:	A. R. Baker	Senior Investigator	SURG NCI	Other:	S. F. Stinson	Pathologist	TP NCI		R. M. Simon	Chief, Biometric Res. Br.	DCT NCI		N. P. Lang	Visiting Scientist	LID NCI		H. J. Rapp	Chief, Lab. Immunobiol.	LIB NCI		M. C. Chappell	Bio. Lab. Tech. (Micro.)	SURG NCI		R. McCauley	Clinical Associate	SURG NCI
PI:	A. R. Baker	Senior Investigator	SURG NCI																											
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	M. C. Chappell	Bio. Lab. Tech. (Micro.)	SURG NCI																											
	R. McCauley	Clinical Associate	SURG NCI																											
COOPERATING UNITS (if any)																														
LAB/BRANCH Surgery Branch																														
SECTION Office of the Chief																														
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205																														
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0																												
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																														
SUMMARY OF WORK (200 words or less - underline keywords) DMH-induced rat colon cancer is being studied as a model for human colorectal malignancy. Neither the primary tumor nor subcutaneous transplant generations of it metastasize frequently to the liver. Recent studies show that portal venous injection of a tumor cell suspension produce numerous foci of hepatic metastatic disease. Work continues on efforts to isolate and develop a RIA for the identified colon tumor-specific factor. Results of studies comparing surgical resection of the primary tumor with intraslesional BCG-CW injection of it failed to show a salutary effect of immunotherapy.																														

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03826-06 SURG						
PERIOD COVERED October 1, 1979, to September 30, 1980								
TITLE OF PROJECT (80 characters or less) Biologic Tumor Markers in the Cells and Sera of Patients with Urologic Cancers								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: N. Javadpour</td> <td style="width: 33%;">Senior Investigator</td> <td style="width: 33%;">SURG NCI</td> </tr> <tr> <td>Other: M. Lippert</td> <td>Guest Worker</td> <td>SURG NCI</td> </tr> </table>			PI: N. Javadpour	Senior Investigator	SURG NCI	Other: M. Lippert	Guest Worker	SURG NCI
PI: N. Javadpour	Senior Investigator	SURG NCI						
Other: M. Lippert	Guest Worker	SURG NCI						
COOPERATING UNITS (if any)								
LAB/BRANCH Surgery Branch								
SECTION: Office of the Chief								
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205								
TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0						
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) Techniques have been developed to localize and quantitate <u>biologic tumor markers</u> in the cells and sera of patients with urologic cancers. These markers include human chorionic gonadotropin, alphafetoprotein, pregnancy specific β_1 glyco-protein (SP₁), different isoenzymes of lactic dehydrogenase (LDH), ABO(H) antigens, and steroid receptors. These markers have been correlated with the histology, grade, stage, and management of patients with urologic cancers. Experimental animal models of cancer induction for the kidney, bladder, prostate, and testis have also been developed. These animal models are being utilized to study the role of cytoreductive surgery, chemotherapy, and immunotherapy of these urologic cancers. Athymic mice have been utilized for xenograft of testicular tumor that produces markers. By utilizing specific antibody labeled with a γ-emitting radioisotope such as ^{131}I, the role of radioimmunodetection and radioimmunotherapy is being investigated in testicular and prostatic cancer.								

Publications:

Javadpour, N., Bagley, D.H., and Zbar, B.: Failure of cryosurgical treatment of experimental intradermal tumors to eradicate microscopic lymph node metastases in guinea pig. J. Natl. Cancer Inst. 62: 1479, 1979.

Woltering, E.A., Emmott, R.C., Javadpour, N., Marx, J., and Brennan, M.F.: ABO(H) cell surface antigens in parathyroid adenoma and hyperplasia. Surg. Forum 30: 107-109, 1979.

Javadpour, N., and Chen, H.: Carboxylterminal RIA in detecting urinary HCG: A sensitive technique. J. Urol., in press.

Javadpour, N., Goldenberg, D.M., and Kim, E.: Radioimmunoassay of metastatic testicular cancer with radioactive antibodies to human chorionic gonadotropin and alphafetoprotein. J. Urol., in press.

Woltering, E.A., Knox, R.D., and Javadpour, N.: Detection of HCG in fresh and formaldehyde fixed testicular tissue: The sensitivity of immunoperoxidase vs. RIA. Urology, in press.

Javadpour, N., Woltering, E.A., and Soares, T.: Simultaneous measurement of tumor cytosol and peripheral serum levels of HCG and AFP. Invest. Urol., in press (June 1980).

Javadpour, N.: Immunocytochemical discordance in localization of pregnancy specific β_1 glycoprotein (SP₁), alphafetoprotein (AFP) and human chorionic gonadotropin in certain testicular cancer. J. Urol., in press (April 1980).

Javadpour, N.: Radioimmunoassay and immunoperoxidase of pregnancy specific β_1 glycoprotein (SP₁) in certain testicular cancer. J. Urol., in press (April 1980).

Emmott, R., Javadpour, N., and Droller, M.J.: The ABO(H) cell surface antigens in carcinoma in situ and nonmalignant lesions of the bladder. J. Urol., in press.

Johnson, H., Flye, M.W., and Javadpour, N.: Serum β_2 microglobulin levels in patients with testicular cancer. Urology, in press.

Danforth, D., and Javadpour, N.: Pressure effects of urinary reflux studies with renal autotransplantation and pyelocystostomy. Urology, (January 1980).

Lippert, M. and Javadpour, N.: Lactic dehydrogenase in monitoring and prognosis of testicular cancer. J. Urol., in press.

Javadpour, N., Utz, M. and Soares, T.: Immunocytochemical discordance in localization of pregnancy specific β , glycoprotein (SP₁), AFP and HCG in certain testicular cancers. Urology, in press.

Bergman, S. and Javadpour, N.: The cell surface antigen A, B, or O(H) as an indicator of malignant potential in Stage A bladder carcinoma. J. Urol. 119: 49, 1978.

Javadpour, N., Hyatt, C.L. and Barone, J.J.: Common antigens found on fetal cells and viral transformed adult prostatic tissue. J. Surg. Oncol. 10:245, 1978.

Javadpour, N., McIntire, K.R. and Waldmann, T.A.: HCG and AFP in sera and tumor cells of patients with seminoma. J. Urol., Dec. 1978.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06652-04 SURG																								
PERIOD COVERED October 1, 1979, to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Studies of Immune Regulation																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">P. H. Sugarbaker</td> <td style="width: 30%;">Senior Investigator</td> <td style="width: 20%;">SURG NCI</td> </tr> <tr> <td></td> <td>W. Matthews, Jr.</td> <td>Research Associate</td> <td>SURG NCI</td> </tr> <tr> <td></td> <td>T. J. Smith</td> <td>Clinical Associate</td> <td>SURG NCI</td> </tr> <tr> <td></td> <td>Y. Roth</td> <td>Clinical Associate</td> <td>SURG NCI</td> </tr> <tr> <td></td> <td>M. Kemeny</td> <td>Research Fellow</td> <td>SURG NCI</td> </tr> <tr> <td></td> <td>F. Gianola</td> <td>Physician's Assistant</td> <td>SURG NCI</td> </tr> </table>			PI:	P. H. Sugarbaker	Senior Investigator	SURG NCI		W. Matthews, Jr.	Research Associate	SURG NCI		T. J. Smith	Clinical Associate	SURG NCI		Y. Roth	Clinical Associate	SURG NCI		M. Kemeny	Research Fellow	SURG NCI		F. Gianola	Physician's Assistant	SURG NCI
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COOPERATING UNITS (if any)																										
LAB/BRANCH Surgery Branch																										
SECTION Office of the Chief																										
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0																								
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SUMMARY OF WORK (200 words or less - underline keywords) The work in this laboratory includes three major projects: (1) Studies involve an assessment of <u>cell-mediated immune responses</u> after the host has responded to a variety of alloantigenic stimulation to the same and other antigens. The significance of this work comes from attempts to understand regulatory mechanisms of cellular immune responses. This work has resulted in the development of a new mechanism of T cell control - the alloantigen elimination hypothesis. (2) Studies on the <u>preferential localization</u> of effector cells to alloantigenic grafts have shown <u>preferential homing</u> of cytotoxic T cells; this homing is abrogated by specific alloantisera; work with the homing properties of <u>in vitro</u> sensitized cells is underway. (3) Studies of <u>local immunotherapy</u> have begun; allogenic and xenogenic systems have been characterized. An adjuvant immunotherapy attack on cancer cells remaining after surgery is planned.																										

Publications:

Sugarbaker, P.H., and Chang, A.E.: Enhancing alloantibody effective during sensitization but not effector phase of skin graft rejection. Transplantation 28: 60-65, 1979.

Chang, A.E., and Sugarbaker, P.H.: Preferential homing of passively transferred T cells into skin allografts. Transplantation 28: 247-252, 1979.

Sugarbaker, P.H., and Chang, A.E.: Uncovered skin grafts in mice. J. Immunol. Techniques 31: 167-175, 1979.

Chang, A.E., and Sugarbaker, P.H.: Abrogation of T cell homing by alloantibody, a new mechanism of immunologic enhancement. Transplantation 29: 127-132, 1980.

Chang, A.E., and Sugarbaker, P.H.: Assessment of T cell function with a skin allograft assay. Transplantation, in press.

Sugarbaker, P.H.: Diminished cellular cytotoxicity in mice hyperimmunized prior to skin grafting. Cell. Immunol., in press.

Sugarbaker, P.H., and Matthews, W.: Early antigen elimination by cytotoxic T cells results in diminished cellular immune response to allogeneic tumor. Cell. Immunol., in press.

Sugarbaker, P.H., and Matthews, W.: Cytotoxic cells suppress in vitro generation of cellular immunity by stimulator cell lysis. Cell. Immunol., in press.

Sugarbaker, P.H., Sugarbaker, S.P., and Pun, P.P.: Characterization of in vivo suppression of syngeneic tumor by allogeneic effector cells. J. Surg. Oncol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06653-04 SURG
PERIOD COVERED October 1, 1979, to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Metabolic Studies in Tumor-Bearing Patients and Animals		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Other:	M. F. Brennan M. E. Burt R. C. Shamberger G. Sloan J. Arbeit M. Maher D. White C. Gorschboth D. Jones M. L. Peters A. Kirkemo A. Saxe	Head, Surg. Metab. Sec. Clinical Associate Clinical Associate Clinical Associate Clinical Associate Clinical Nurse Computer Analyst Medical Technologist Biologist Clinical Associate Clinical Associate IPA, Univ. of California
SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI SURG NCI		
COOPERATING UNITS (if any)		
LAB/BRANCH Surgery Branch		
SECTION Surgical Metabolism Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 00.0	PROFESSIONAL: 0.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The Surgery Branch is vigorously involved in the assessment of the efficacy of <u>total parenteral nutrition</u> as a means of nutritional support of the cancer-bearing host. These studies utilize methodologies employing stable isotopes and labelled substrates to examine glucose and amino acid metabolism. Animal models are utilized for examination of gluconeogenesis and glucose kinetics. Particular efforts are directed at examining methods of host preservation and metabolic cost of antineoplastic therapy. Continued effort is being made in the elucidation of function of fresh and cryopreserved parathyroid and other endocrine tissues.		

Publications:

Goodgame, J.T., Lowry, S.F., Reilly, J.J., Jones, D.C., and Brennan, M.F.: Nutritional manipulations and tumor growth: I. The effect of starvation. Am. J. Clin. Nutr. 32: 2277-2284, 1979.

Norton, J.A., Kahn, C.R., Gorschboth, C., and Brennan, M.F.: Concomitant reversibility of skin rash and amino acid deficiency by parenteral amino acids and glucose in a patient with a malignant glucagonoma. Ann. Int. Med. 91: 213-215, 1979.

Norton, J.A., Popp, M.B., and Brennan, M.F.: Comparison of ^{15}N flux and nitrogen balance with carcass analysis during oral and IV nutrition. Surg. Forum 30: 55-57, 1979.

Goodgame, J.T., Lowry, S.F., and Brennan, M.F.: Nutritional manipulations and tumor growth. II. The effects of intravenous feeding. Am. J. Clin. Nutr. 32: 2285-2294, 1979.

Lowry, S.F., Norton, J.A., and Brennan, M.F.: Glucose turnover and gluconeogenesis during hypocaloric glucose infusion in tumor-bearing animals. J. Natl. Cancer Inst. 64: 291-296, 1980.

Burt, M.E., Norton, J.A., and Brennan, M.F.: The human tumor-bearing limb: An ex vivo model. Surgery 87: 123-132, 1980.

Devereux, D.F., Thistlethwaite, P., Thibault, L., and Brennan, M.F.: The effects of tumor-bearing and protein-depletion on wound breaking strength in the rat. J. Surg. Res., in press.

Devereux, D.F., Triche, T., Webber, B., and Brennan, M.F.: Etiology of Adriamycin wound breaking strength in rats - A study by light and electron microscopy, induction of collagen maturation and hydroxyproline assay. Cancer, in press.

Norton, J.A., Burt, M.E., and Brennan, M.F.: In vivo utilization of substrate by human sarcoma bearing limbs. Cancer, in press.

Devereux, D.F., Kent, H., and Brennan, M.F.: Time dependent effects of Adriamycin and x-ray therapy on wound healing in the rat. Cancer, in press.

Burt, M.E., Arbeit, J., and Brennan, M.F.: Chronic arterial and venous access in the unrestrained rat. J. Appl. Physiol., in press.

Sloan, G.M., Norton, J.A., and Brennan, M.F.: Influence of diabetes mellitus and insulin treatment on protein turnover in the rat. J. Surg. Res., in press.

Burt, M.E., Lowry, S.F., Gorschboth, C., and Brennan, M.F.: Metabolic alterations in a noncachectic animal tumor system. Cancer, in press.

Brennan, M.F., and Burt, M.E.: Nitrogen metabolism in cancer. Cancer Treat. Rep., in press.

Popp, M.B., Morrison, S.D., and Brennan, M.F.: Total parenteral nutrition in a methylcholanthrene-induced rat sarcoma model. Cancer Treat. Rep., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06654-03 SURG

PERIOD COVERED

October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies in Malignant Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. F. Sindelar	Senior Investigator	SURG NCI
Other:	C. Kurman	Microbiologist	SURG NCI
	A. Dresdale	Clinical Associate	SURG NCI
	J. Tepper	Senior Investigator	ROB NCI
	E. Travis	Senior Investigator	ROB NCI

COOPERATING UNITS (if any)

Radiation Oncology Branch

LAB/BRANCH

Surgery Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with sarcomas are studied for evidence of serum reactivity against tumor-specific determinants expressed on both fresh and cultured syngeneic and allogeneic tumor cells using staining techniques of immunofluorescence and immunoperoxidase. Tissue-specific antigens have been isolated and are being investigated for possible use in immunotherapy of carcinogen-induced pancreatic carcinoma carried in inbred hamsters. Intraoperative radiotherapy is evaluated in dogs to determine responses of both normal and surgically-manipulated tissues to direct single-dose electron-beam irradiation in approaches to adapt operative radiotherapy to the treatment of human abdominal malignancies.

Publications:

Hadley, N.A., and Sindelar, W.F.: Antifetal and tumor-specific reactivity in human osteosarcoma. Surg. Forum 30: 122-124, 1979.

Rosenberg, S.A., Tondreau, S.C., Shoffer, P.C., Hyatt, C., Brown, J.M., Thorpe, W., and Sindelar, W.: Serologic analysis of human tumor antigens. II. Reactivity of sera from eleven osteogenic sarcoma patients against autologous and allogeneic tumor cells. Surgery 86: 258-265, 1979.

Sindelar, W.F., and Mason, G.R.: Aortocystoduodenal fistula. Rare complication of pancreatic pseudocyst. Arch. Surg. 114: 953-955, 1979.

Auda, S.P., and Sindelar, W.F.: Bipedicle transverse abdominal cutaneofascial flap for reconstruction of lower thoracic wall defects. J. Surg. Oncol., in press.

Rosenberg, S.A., and Sindelar, W.F.: Surgery and Adjuvant Radiation-Chemo-Immunotherapy in Soft Tissue Sarcomas: Result of Treatment at the National Cancer Institute. In Cleton, F.J., Muggia, F.M., van Oosterom, A.T. (Eds.): Advances in the Treatment of Ovarian and Testicular Cancer and Sarcomas. New York, Academic Press, in press.

Rosenberg, S.A., and Sindelar, W.F.: Surgery and Adjuvant Chemo-Immunotherapy in Osteosarcomas: Review of Treatment at the National Cancer Institute. In Cleton, F.J., Muggia, F.M., van Oosterom, A.T. (Eds.): Advances in the Treatment of Ovarian and Testicular Cancer and Sarcomas. New York, Academic Press, in press.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE BALTIMORE CANCER RESEARCH PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1979 through September 30, 1980

The Baltimore Cancer Research Program has completed its 5th year at the University of Maryland Hospital. The Clinical Branch has provided for approximately 11,000 inpatient days and 14,000 outpatient visits.

The Clinical Branch continues to pursue Phase I and II drug testing. New drugs, such as AMSA, pentamethylmelamine, thymidine, anthracenedione and aclacinomycin have been studied in patients.

Phase III studies of hyperthermia and chemotherapy for patients with metastatic sarcoma and lung cancer are in progress. A major objective of these studies is to determine whether hyperthermia can alter efficacy on toxicity of cancer chemotherapeutic agents to the advantage of the patient.

Phase III studies of daunorubicin or adriamycin and cytosine arabinoside in adult acute nonlymphocytic leukemia have demonstrated a 70% complete response rate. Current studies in adult acute leukemia are evaluating various maintenance schemes, including late intensification therapy, splenectomy, and immunotherapy with neuraminidase-treated leukemic cells. Combined modality studies of Hodgkin's disease continue to show significant advantage for radiotherapy-chemotherapy in patients with stage IIIA or with early stages associated with "E" substage of the lung. Evaluation of a recently completed Hodgkin's disease study comparing chemotherapy with radiotherapy plus chemotherapy shows that MOPP chemotherapy and radiotherapy are equally effective in producing complete responses in patients with stage II-IIIa Hodgkin's disease. These results have led to a joint study with other DCT clinical branches of early Hodgkin's disease which is testing the relative merits of radiotherapy, chemotherapy, and combined modality treatment. In addition, we have initiated a combination chemotherapy study of advanced Hodgkin's disease involving all DCT clinical branches.

Evaluation of over 100 patients with oat cell carcinoma of the lung who have received combination chemotherapy alone has revealed interesting results. The combination of cyclophosphamide, adriamycin, and VP16-213 appears to yield results comparable to other studies in which chemotherapy and radiotherapy were used together. A small fraction of patients treated on our chemotherapy alone studies are disease free and off all treatment for 6 to 18 months thus far.

Several active new drug combinations have been identified for metastatic breast, ovarian, cervical and bladder cancer.

Programs of the Section of Infection Research and Research Microbiology have continued in five major areas, namely surveillance, epidemiology, diagnosis, treatment, and prevention. This work has led to the identification of refined empiric antibiotic combinations with which to treat febrile granulocytopenic cancer patients more and more successfully.

Summary Report
Associate Director, BCRP, DCT, NCI (cont'd)

Studies with autologous frozen platelets in acute leukemia patients without compatible donors have shown them to be life saving. This study plus the prophylactic granulocyte transfusion study represent major contributions from our Cell Component Therapy Unit to our program and to the treatment of leukemia and cancer patients at large.

The Laboratory of Clinical Biochemistry general areas of investigation include studies of drug free radical pharmacology, the study of human metabolic pathways for new chemotherapeutic agents, the study of metal-anthracycline interaction and its relationship to anthracycline-related cardiotoxicity and anthracycline mechanism of action, the study of cellular control mechanisms that affect cell growth and differentiation, the study and identification of new biologic response modifiers, and, finally, certain immunological studies in conjunction with the Clinical Oncology Branch. All new drug clinical evaluations performed by that branch are correlated with LCB laboratory investigations. Clinical evaluation of new modalities of treatment, such as hyperthermia, is done in conjunction with the LCB. The laboratory is responsible for defining certain drug metabolic alterations that result from such new treatment modalities. Thus, the LCB performs basic scientific work related to drug pharmacology and metabolism in addition to sharing in clinical investigations with the COB.

A major reorganization of scientific priorities of the Laboratory of Molecular Biology has resulted in that laboratory assuming a larger share of the BCRP mission within the scope of the DCT program. Recently activated studies of this laboratory are designed to shed light on the mechanism of action of interferon and several important observations concerning interferon and nuclease interactions have been made. Technology developed in this laboratory has allowed for the identification of new nuclease species which may be disease-specific. Studies are underway in collaboration with the Clinical Oncology Branch to determine the significance of these enzymes and their potential usefulness as biomarkers. Studies of functions of viral proteins have uncovered new data potentially useful in understanding viral oncogenesis. Various interactions between Rauscher leukemia virus reverse transcriptase and P30 and P12 protein have been identified which may be important in determining the fidelity of transcription of viral DNA and retroviral replication. A new oncogenic virus has been isolated from AKR mice which appears to be a recombinant between ectotropic and xenotropic viruses. The presence of portions of the herpes simplex virus type 2 gene has been detected in leukemic cells from some patients and in tumor cells from patients with cervical carcinoma. These exciting studies are continuing in collaboration with the Clinical Oncology Branch.

Close scientific interaction among the various DCT branches in Bethesda and Baltimore continues. To date, this cooperation has led to joint lymphoma and leukemia protocols and to joint Phase I studies of drugs considered of prime importance to the DCT. This cooperation has led to better utilization of clinical material on both campuses and to faster clinical results derived from larger numbers of patients that previously were not possible for the DCT intramural program.

SUMMARY REPORT
CLINICAL ONCOLOGY BRANCH
BALTIMORE CANCER RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
October 1, 1979 through September 30, 1980

The Clinical Oncology Branch has had another productive year. Phase I studies of pentamethylmelamine and anthracenedione have been completed, and a Phase I study of aclacinomycin has been started. Phase II studies completed or nearing completion include AMSA in renal cell and ovarian cancer, and melanoma; methyl GAG in renal cell cancer; and IV thioguanine in metastatic colon cancer. A Phase II study of thymidine and cytosine arabinoside for relapsed adult acute nonlymphocytic leukemia patients has yielded a 50 percent response rate. Phase III studies of anthracycline antibiotics in conjunction with cytosine arabinoside in acute nonlymphocytic leukemia have continued to yield complete response rates of approximately 70% and a 4 year disease free survival rate of 25%. Allogeneic neuraminidase-treated leukemic blast cells are under investigation as immunotherapy in these patients. Early results suggest remission duration prolongation with this treatment. The interferon inducer Poly IC:LC is under investigation in relapsed acute leukemia adult patients, and antileukemic effect has been demonstrated.

Hodgkin's disease treatment research continues to be a major interest of the Branch. Phase IV combined modality studies continue to show significant advantage over radiotherapy in patients with extensive Stage IIIA disease, and in patients with E substage disease (lung). Some of this work has recently been confirmed by a joint BCRP, Harvard, Vanderbilt, and University of Chicago study which has just been published. New Hodgkin's disease studies undertaken jointly by the Clinical Oncology Branch, BCRP and the Radiation Oncology and Medicine Branches, COP are well underway, and are designed to further define the optimal therapy for patients with Hodgkin's disease of all stages. Several more years of patient accrual will be necessary before meaningful results are obtained.

Studies of cyclophosphamide, adriamycin, and VP16-213 in oat cell carcinoma of the lung have resulted in a 90% response rate. Approximately 15 percent of patients so treated remain disease free off all therapy for 6-18 months after treatment thus far. It is hoped that some of these patients may actually be cured of their disease with chemotherapy alone.

Phase I and II studies in gynecological malignancies have recently been possible due to collaboration of the Department of Obstetrics and Gynecology of the University of Maryland Hospital with the Branch.

A Phase III study of vinblastine and Mitomycin C in advanced refractory metastatic carcinoma of the breast has shown that treatment to be highly active. An international study of advanced breast cancer conducted under the auspices of Cancer and Leukemia Group B and chaired by members of the Branch has demonstrated the benefit of the addition of adriamycin to conventional treatment.

Summary Report
Clinical Oncology Branch, BCRP (cont'd)

Phase III studies of hyperthermia and chemotherapy for lung cancer and metastatic sarcoma are underway. Twenty patients have been treated and pharmacokinetic data have been collected, but it is too early to fully evaluate the results.

In the Cell Component Therapy Section, studies of frozen autologous platelet transfusions for acute leukemia patients continue to be rewarding. We have transfused platelets, frozen at liquid nitrogen temperature, for more than 4 years back to the donor at a time when he was thrombocytopenic due to leukemic relapse. The transfusions have yielded increments suggesting approximately a 50% recovery rate of frozen platelets. A prophylactic granulocyte transfusion study has been completed. Newer methods of cryopreservation are under study. Radiolabelled granulocytes have been utilized in an effort to determine the fate of transfused granulocytes. This Section continues to supply human leukemic cells for study by several DCT laboratory branches in Baltimore and Bethesda.

Programs of the Sections of Infection Research and Research Microbiology involve five major areas: surveillance, epidemiology, diagnosis, treatment, and prevention. Treatment studies have included evaluations of new antimicrobials, both in the laboratory and in the clinic. New drugs, including piperacillin, mezlocillin and trimethoprim, have been evaluated in vitro and in the clinic. Infection prevention has emphasized refinements of antimicrobial prophylaxis. The opening of a new 4 bed laminar air flow unit has allowed studies of relapsed leukemia patients, and patients over the age of 65 in this setting. Early results suggest significant infection prevention by these units in poor risk leukemia patients.

Biologic markers of malignant disease such as terminal transferase and reverse transcriptase have been studied in some detail and the data correlated with the type and extent of hematologic malignancy. New, previously unreported biomarkers are under study in oat cell carcinoma jointly by the Branch and the Frederick Cancer Research Center.

The massive research data accumulated by the Branch has been prepared for analysis on the PROMIS System. Physicians, nurses, pharmacists, and other related health care personnel take part in the input and the retrieval of this data. This system is undergoing major expansion as a result of the arrival of our own computer and terminal equipment.

Participation in oncology programs at the two medical schools in Baltimore and at other hospitals around the community has provided a source of patient material for our studies and has enabled us to bring to the practicing and academic physician in the community, the impact of the therapeutic advances resulting from the COB program.

To extend the Computerized Problem-Oriented Medical Information System (PROMIS) to the cancer treatment area to allow the development of structured input ("cancer displays") for coupling learning with doing for all personnel who care for individual cancer patients entered on problem specific plans (cancer treatment research protocols).

Methods Employed:

1. The computer terminal being used for this purpose is a touch-sensitive cathode ray tube display device similar to those in use in Burlington, Vermont, where the computer is located. BCRP has been responsible for the installation since February, 1972. All programming and operation of the computer system have been done at the University of Vermont.
2. The basic components of the remote terminal at the BCRP are the same as the local terminals in Burlington, Vermont: display screen, display memory, keyboard, touch screen and communication link to the computer in the PROMIS Laboratory, Burlington, Vermont.

Results:

1. The BCRP staff has developed antineoplastic drug displays which have been integrated with the displays already on file in Burlington, Vermont, as part of the PROMIS system. The drug information sequences have been updated and new sequences have been added. Platinum drug displays have been added. Update and revision of the pyrimidine antagonists are completed. Reference citations using new citation tables are complete for alkylating agents and are in progress for antimetabolites as they are being updated. Monitoring information for all antineoplastic agents is continuing. All alkylating agents have been audited and updated. Drug dosage information structures were expanded to include 1) general information for planning drug dosages; 2) technique to prepare drugs and 3) technique to administer drugs.
2. Problem formulation sequences for breast cancer and lung cancer were revised and expanded to match current standard terminology. Lymphoma problem formulation sequences were updated to match current standards. Problem formulation sequences for colon cancer, sarcomas, ovarian, testicular and melanoma are also being updated.
3. Problem specific plans for small cell carcinoma of the lung have been completed including BCRP protocol #7705 which will serve as a model for other protocols that will be updated and added. A BCRP protocol for acute lymphocytic leukemia has been added to the system. The facility for adding additional protocols being developed does not require any additional programming effort.

4. Bone marrow reporting displays have been completed.
5. The entire systems package has been shipped to BCRP and testing and debugging has begun in conjunction with the Intragency Agreement with the National Center for Health Services Research (Y01 CM 80109).

The computerized displays for the logic and action of treating cancer problems are defined by the creation and sequencing of the computer display pages or frames. These displays provide an automatic connective device between the four phases of medical action of the Problem Oriented Medical Record. The problem formulation sequence determines the specific plan and it is that plan which is in part a protocol or series of protocols for treating a specific cancer problem. The protocol couples the user to the protocol specific antineoplastic drug displays which provide feedback loops to the patient's problem list and outstanding orders. It is through the use of these explicit informational and directional displays that the user is provided guidance while gathering information and treating patients' cancer problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06263 09 COB
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PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Protocol for Staging and Treatment of Non-Hodgkin's Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard S. Kaplan, MD.	Senior Investigator	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
Robert Slawson, M.D. Division Radiation Therapy
University of Maryland Hospital, Baltimore, Maryland 21201

LAB/BRANCH
Clinical Oncology Branch
SECTION

Office of the Chief
INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

At the time of presentation, non-Hodgkin's lymphomas are frequently disseminated. There exists, however, a number of patients with localized disease following completion of all clinical and pathologic staging procedures. It is in this group of patients that radiotherapy is most useful. However, the question of whether adjuvant chemotherapy following radiation therapy is of any benefit is as yet unanswered. Patients who are found to have pathologically Stage I and II non-Hodgkin's lymphomas following a variety of staging procedures including staging laparotomy were randomized to receive either extended field irradiation alone, or extended field irradiation followed by six courses of chemotherapy using Cytosan, vincristine and prednisone. Fifteen patients whose staging laparotomies were entirely negative have been randomized to one or the other of the treatment arms. Analysis of staging data is underway. No conclusion can be drawn as yet as to the value of adjuvant chemotherapy.

1. To assess the value of surgical staging in patients with apparently localized non-hodgkin's lymphomas.
2. To determine whether adjuvant chemotherapy will prolong remissions in patients with pathologically confirmed localized lymphomas treated with radiation therapy.

Patients with non-hodgkin's malignant lymphomas underwent a variety of staging procedures. Those whose disease appeared clinically localized following non-invasive procedures underwent staging laparotomy to detect occult foci of intra-abdominal disease. Patients who were found to be pathologic Stage I or II were then randomized to receive (a) radiation therapy alone, or (b) radiation therapy followed by six courses of combination chemotherapy using Cytoxan, vincristine and prednisone. Patients who were found to have advanced disease by these staging procedures were treated with chemotherapy alone on a different protocol. Of the patients undergoing all staging procedures, there have been 15 who have been found to have pathologic Stage I or II. At this time, there are too few patients in either of the study arms and follow-up time is too short to determine the value of adjuvant chemotherapy. The staging data are being analyzed.

PROPOSED COURSE: This study will be terminated but entered patients will continue to be followed.

PUBLICATIONS:

1. Diggs, C.H., Wiernik, P.H. and Ostrow, S.S.: Nodular Lymphoma: Prolongation of Survival by Complete remission. Cancer Clinical Trials (In press) 1980.
2. DeVita, V.T., Glatstein, E.J., Young, R.C., Hubbard, S.M., Simon, R.M., Ziegler, J.L. and Wiernik, P.H.: Changing Concepts: The Lymphomas. In Jones, S.E. and Salmon, S.E. (eds). Adjuvant Therapy of Cancer II, Grune & Stratton, New York 1979, pp 179-190.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 CM 06277-09 COB	
PERIOD COVERED October 1, 1979 through September 30, 1980					
TITLE OF PROJECT (80 characters or less) Chemotherapy of Adult Acute Leukemia					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI: Peter H. Wiernik, M.D.		Chief, Clinical Oncology Branch		COB NCI	
Others: Stephen Schirpf, M.D.		Head, Section of Infection Research		COB NCI	
Charles Schiffer, M.D.		Head, Cell Component Therapy Section		COB NCI	
Dean Brenner		Clinical Associate		COB NCI	
Joseph Aisner, M.D.		Head, Section of Medical Oncology		COB NCI	
Kerry Van Sloten, R.N.				COB NCI	
Susan Markus, R.N.				COB NCI	
Patricia Duffy, R.N.				COB NCI	
Viola Mae Young, Ph.D.		Head, Section of Microbiology		COB NCI	
Prem Sarin, Ph.D.		Deputy Chief, Laboratory of Tumor Cell Biology		LTCB NCI	
Theodore Breitman, Ph.D.		Senior Investigator		LTCB NCI	
COOPERATING UNITS (if any) Laboratory of Tumor Cell Biology, DCT, NCI and Laboratory of Clinical Biochemistry, BCRP, NCI					
LAB/BRANCH Clinical Oncology Branch					
SECTION Office of the Chief					
INSTITUTE AND LOCATION NCI, NIH Baltimore, Maryland 21201					
TOTAL MANYEARS: 6.5		PROFESSIONAL: 6.5		OTHER: 0	
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) A 7-day continuous intravenous infusion of cytosine arabinoside plus a 3-day schedule of intermittent daily doses of Adriamycin has been shown to be as effective chemotherapy for the induction of remission in adults with <u>acute nonlymphocytic leukemia</u> as the combination of cytosine arabinoside with daunorubicin. A complete remission rate of 65% has been obtained with either combination. However, the adriamycin combination has been shown to be more toxic and to require more careful dosage adjustment and monitoring of blood levels. At present it is too early to discern whether the addition of either neuraminidase-treated allogeneic myeloblasts or splenectomy to chemotherapy during remission maintenance treatment enhances the duration of remission or survival of patients. Reverse transcriptase has been identified in the bone marrow cells of leukemia patients prior to treatment and in morphologically normal peripheral cells during complete remission. This may suggest that morphologically and functionally normal granulocytes in the peripheral blood of leukemia patients in complete remission may actually be derived from leukemic cells.					

I. Therapy:

A. Remission Induction.

Our previous investigation demonstrated that a 7-day continuous intravenous infusion of cytosine arabinoside plus a 3-day schedule of intermittent daily doses of daunorubicin is a highly effective chemotherapy for induction of remission in adults with acute non-lymphocytic leukemia. A complete remission rate of 65% was obtained with a median remission duration of 6 months and a median survival from the initiation of therapy of 12 months. Analysis of factors which may have affected the duration of remission demonstrated that younger patients, patients who were uninfected at the time of admission, and female patients had significantly higher remission induction rates and overall survival than did their counterparts. Half of the patients in this study were also treated with an immuno-reconstitutive agent, levamisole, which did not appear to augment either remission induction rate or remission duration. Although survival after relapse in patients who received levamisole was somewhat longer than survival in the group of patients not receiving levamisole, the reason for this effect has not been completely elucidated.

Forty-five patients have been entered into a new study utilizing a 7-day continuous infusion of cytosine arabinoside with a 3-day intermittent dosage regimen using Adriamycin instead of daunorubicin for induction therapy. Of 44 evaluable patients, 65% have achieved complete remission with a median duration of remission of 11 months. Toxicity with this induction regimen appears to be greater than that seen with cytosine arabinoside plus daunorubicin. For this reason, daunorubicin has been substituted for adriamycin in our current study. Sixty-five patients have accrued to the current study and a 65% complete response rate has been observed. The purpose of the current study is to provide an adequate number of complete responders for a 3 arm randomized study of maintenance therapy.

B. Maintenance Therapy for Remission in Adults with Acute Non-Lymphocytic Leukemia.

Following the attainment of remission, patients are currently being randomly assigned to receive either cytosine arabinoside and 6-thioguanine given to hypoplasia intermittently, the same chemotherapy with the addition of neuraminidase-treated allogeneic myeloblasts, or the same chemotherapy plus splenectomy. In our previous study, 7 patients received the late intensification phase of maintenance with cytosine arabinoside and thioguanine. Although 4 of these patients have now relapsed from 2 to 4 years after remission induction, 3 patients continue in their first complete remission more than 4 years since the initiation of treatment. Because of this observation, we are currently using intensive intermittent courses of cytosine arabinoside and thioguanine as the control arm of this study. Another third of the patients who achieve remission are assigned to receive this same chemotherapy plus neuraminidase-treated myeloblasts in an attempt to immunologically stimulate patients who have achieved complete

remission to destroy or suppress the development of additional leukemic blasts. This study design is based on the successful trials of this type of immune stimulation in delaying the development of spontaneous AKR lymphoma in mice, as well as a prospectively randomized trial of immunotherapy begun in 1973 by Bekesi and Holland which suggests that there is a highly significant prolongation of both median remission duration and survival in a group of patients receiving neuraminidase-treated cells as immunotherapy in addition to their chemotherapy. Sixteen patients are currently under treatment with this regimen. Another third of the patients who achieve complete remission undergo splenectomy after remission attainment following which they are also treated with chemotherapy identical to that of the patients above. This is based on reports that splenectomy significantly delays the onset of leukemia in the AKR mouse model, and also on anecdotal reports of long-term survival in splenectomized adult patients with acute nonlymphocytic leukemia. An additional study done by Fleming and associates also suggested that there was an enhanced remission duration and survival in children with acute myelogenous leukemia who underwent splenectomy plus chemotherapy during maintenance compared with children who are not splenectomized. In 12 of the 14 patients, there was also histologic evidence of residual leukemia in the spleens which were removed. Seventeen patients have been randomized to this arm.

It is too early to comment upon the success of the maintenance therapy of this study; however, it is projected that approximately 100 patients need to be entered onto study and it is projected that the study will therefore continue to accrue patients for at least two more years.

11. Biochemical Indicators of Disease Activity in Acute Non-Lymphocytic Leukemia:

A. Serum and Urine Muramidase Studies

We continue to confirm the utility of serum muramidase levels in differentiating between acute monocytic, myelomonocytic, and myelocytic leukemia. In addition, we continue to observe that serum muramidase levels accurately reflect disease activity in a large proportion of patients with acute non-lymphocytic leukemia. It has also become evident that patients with initially elevated serum muramidase levels who achieve complete remission and normal serum muramidase levels have a longer complete remission than those who achieve hematologic and clinical remission but maintain elevated serum muramidase levels.

B. Carcinoembryonic Antigen

We have observed that approximately 30% of adult acute non-lymphocytic leukemia or acute lymphocytic leukemia patients have abnormal levels of carcinoembryonic antigen in their serum. Unfortunately, these levels do not correlate well with disease activity. Most patients undergoing therapy have a rise for weeks or months in their plasma CEA whether or not they respond to therapy. In addition, when patients relapse the presence or absence of

CEA in their plasma at that time does not correlate with findings at the time of presentation. CEA levels in these patients fluctuate with hepatic function.

C. Serum Immunoglobulin Concentrations in Patients with Acute Non-Lymphocytic Leukemia

We have measured quantitatively serum IgA, IgD, IgE, and IgM in patients with acute non-lymphocytic leukemia prior to therapy, during therapy, during remission and relapse. Most patients with acute non-lymphocytic leukemia present with decreased levels of IgG and elevated levels of IgM in their serum. When complete remission is achieved these levels become normal.

D. Cell-Mediated Immunity in Acute Non-Lymphocytic Leukemia

Cell-mediated immunity in acute non-lymphocytic leukemia, either at initial presentation or in subsequent relapse, was determined among 98 patients by recall-antigen skin testing and in 67 patients by attempted sensitization and challenge with dinitrochlorobenzene (DNCB). The leukemic patient's subsequent prognosis in terms of attainment of complete remission of his leukemia, duration of remission and survival does not correlate with the pre-therapy demonstration of intact delayed cutaneous hypersensitivity.

E. Deoxynucleotide Terminal Transferase Studies

This DNA polymerase, originally found in normal thymic cells, has subsequently been identified in relatively high concentrations in the blast cells of most patients with childhood and adult acute lymphocytic leukemia. It has also been reported to be present in high concentrations in blasts from a large minority of patients with chronic myelogenous leukemia in blast crisis, as well as a small fraction of patients with otherwise morphologically classified acute non-lymphocytic leukemia in whom histochemistry and Romanowsky staining are equivocal. Because of the suggestion that this enzyme may provide a means for classifying leukemic cells (and hence stratifying treatment for patients) in addition to the classical morphologic and clinical criteria, blast cells of all new patients with acute leukemia are being assayed for their terminal transferase activity in addition to more routine histologic studies.

Publications:

1. Chang, P.: Adult Leukemia. In Hartmann, P.M. (Ed.): Guide to Hematologic Disorders, New York, Grune & Stratton, 1980, pp. 213-235.
2. Ross, D.D., Wiernik, P.H., Sarin, P.S., Whang-Peng, J.: Loss of terminal deoxynucleotidyl transferase (TdT) activity as a predictor of emergence of resistance to chemotherapy in a case of chronic myelogenous leukemia in blast crisis. Cancer 44:1566-1570, 1979.

3. Wiernik, P.H., Edwards, L.S., Sarin, P.: Terminal deoxynucleotidyl transferase activity in adult acute leukemia. In Neth, R., Hofschneider, P-H, Mannweiler, K., (Eds.): Modern Trends in Human Leukemia III, Heidelberg, Springer Verlag, 1979, pp. 125-130.
4. Wiernik, P.H., Jones, B., Weinberg, V., Holland, J.F.: Present day results in young patients with acute nonlymphocytic leukemia (ANLL). The Cancer and Leukemia Group B Experience. In Mandelli, F. (Ed.): Therapy of Acute Leukemias, Rome, Lombardo Editore, 1979, pp. 430-443.
5. Wiernik, P.H., Glidewell, O.J., Hoagland, H.C., Brunner, K.W., Spurr, C.L., Cuttner, J., Silver, R.T., Carey, R.W., DelDuca, V., Kung, F.H., Holland, J.F.: A comparative trial of daunorubicin, cytosine arabinoside, and thioguanine, and a combination of the three agents for the treatment of acute myelocytic leukemia. Med. Ped. Oncol. 6:261-277, 1979.
6. Muggia, F.M., Chiuten, D.F., Von Hoff, D.D., Rozencweig, M., Wiernik, P.H.: New drugs against human acute leukemia. In Crowther, D.G. (Ed.): Advances in Medical Oncology, Research and Education, Vol. 7: Leukemia and Non-Hodgkin Lymphoma, New York, Pergamon Press, 1979, pp. 55-62.
7. Wiernik, P.H.: Polycythemia. In Hartman, P.M. (Ed.): Guide to Hematologic Disorders, New York, Grune & Stratton, Inc., 1980, pp. 181-190.
8. Chang, P., Wiernik, P.H.: The Leukemias. In Tice, F. (Ed.): Practice of Medicine, Hagerstown, Harper & Row, 1978.
9. Chang, P., Wiernik, P.H.: Chapter 12: Leukemia. In Spivak, J.L. (Ed.): Fundamentals of Clinical Hematology, Hagerstown, Harper & Row, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06293-09 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Teaching and Consultation Service in Medical Oncology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Peter H. Wiernik, M.D. Other: Joseph Aisner, M.D. Stephen C. Schimpff, M.D. Charles A. Schiffer, M.D. David A. Van Echo, M.D. Robert J. Esterhay, Jr., M.D. Richard M. Kaplan, M.D. Richard D. Leavitt, M.D.	Chief, Clinical Oncology Branch Head, Section of Medical Oncology Head, Infection Research Section Head, Cell Component Therapy Section Senior Investigator Senior Investigator Senior Investigator Investigator	COB NCI COB NCI COB NCI COB NCI COB NCI COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) University of Maryland School of Medicine, Johns Hopkins University School of Medicine, Greater Baltimore Medical Center, Good Samaritan Hospital, Baltimore; Veterans Administration Hospital, Baltimore; Franklin Square Hospital, Baltimore		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH Baltimore, Maryland 21201		
TOTAL MANYEARS: 3	PROFESSIONAL: 3	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) In order to improve the clinical approach to various cancers, dissemination of information concerning the latest techniques, diagnosis and therapy is of paramount importance. By providing exposure and training of potential physicians early in the course of their education, such as during their student or house officer years, such information will be brought to the community in an orderly fashion and will preclude the formation of prejudicial opinions about the treatment of malignancies. With the provision of a consultative service to the various cooperating units, we have the opportunity to educate the patients' physicians regarding modern techniques of management of the various neoplastic diseases. These consultative and teaching functions also elevate the level of care available to the patient with cancer in the metropolitan area.		

The objective of this project is to acquaint physicians in training (medical students and house officers) with the theory and practice of medical oncology and how to apply the modalities of chemotherapy, radiotherapy and surgery in the management of neoplastic diseases. In addition, the object of this project is to provide consultative services for difficult patients with neoplastic diseases for other physicians in the Baltimore metropolitan area.

Medical students from the University of Maryland are assigned to this service during their second year on a regular basis for physical diagnosis. In addition, students from the University of Maryland as well as Johns Hopkins rotate through the BCRP during elective periods for a clinical clerkship in medical oncology. In addition, house officers from the Greater Baltimore Medical Center and Good Samaritan Hospital have been assigned here for elective periods of their house officer years. Assistant residents in medicine from the University of Maryland rotate through the Clinical Oncology Branch on a regular basis. In addition, physicians from our Program provide lectures and discussions of difficult cases at many of the cooperating units.

Physicians in the community refer patients here for outpatient consultation. If the patient meets the qualifications for a BCRP protocol study, he or she is accepted and offered the opportunity to be admitted to our Program. If not, the patient is completely evaluated as an outpatient and returned to his physician with specific recommendations for further therapy.

The rotation through the BCRP for medical students and house officers has resulted in a wider acceptance of medical oncology as a sub-specialty of internal medicine at the University of Maryland and elsewhere in the Baltimore community. Because of the contact with house officers and local physicians it has become more accepted that patients with neoplastic diseases require special care based on knowledge derived from specialty training. Some local private physicians have suggested that the general care of patients with neoplastic disease in the community has advanced because of our consultative services.

It is of paramount importance that information concerning the latest techniques in the diagnosis and treatment of cancer patients be disseminated to the medical community. By training potential physicians early during their student to house officer years, such information will be brought to the community in a more orderly and useful way and will preclude the formation of unfounded prejudices. By seeing patients in consultation, we have the opportunity to educate the patient's physician regarding modern techniques of management for any given neoplastic disease. By providing these teaching functions, we engage in and are of significant import in elevating the level of care available to patients with cancer in this area.

OBJECTIVES:

1. To compile, update, and retrieve from a data base of medically relevant information on cancer patients that is centralized, readily accessible and maintained by a single non-technical individual.
2. To generate a daily report on all inpatients and their locations, along with their vital statistics and current therapy data to be distributed to all involved in the tasks of clinical research, patient care and administration.
3. To develop flexible data retrieval programs which generate a variety of useful patient summaries keyed on any of several patient parameters.

METHODS EMPLOYED:

1. Information is compiled and recorded daily in a data input log by a data processor.
2. A computer program accepts the input datum from the secretary, checks for a logical content and accuracy, and records it on the data base.
3. A second computer program generates a Daily Census Report which reflects the updated status of the data base.
4. The report is then distributed to the scientific and professional staff, remote contracting laboratories and administrative office.
5. The programs and data base are executed and stored on a commercial time-sharing service.
6. Mass memory disc files and remote terminals form the storage and access mediums.

The Daily Census Report has been utilized by all sections and services of the Cancer Program.

1. Admissions Officer: used to assign new patients to their physicians and to distribute the patient load evenly among the physicians.
2. Clinical Associates: used at evening sign-out rounds as a checklist of patients with special problems that may require extra care overnight.

3. Clinical Oncology Branch: the master retrieval programs are most useful to the physician performing a chart review to evaluate treatment protocols and who requires listings of the patient population which are sorted by status, cancer diagnosis, etc.
4. Clinical Research Pharmacy Service: used to fill the unit drug cart patient trays.
5. Sections of Microbiology and Infection Research: provides the laboratory staff with relevant information on patients whose cultures are being analyzed. The permanent records of all beds a patient has occupied provide a useful epidemiological tool in detecting the spread of infectious organisms. The data base is also accessed by computerized microbiology reporting systems at the BCRP (see Project Report Z01 CM 06039 07 BCRP).
6. A general purpose information management software library now performs data base manipulations and retrievals which previously required the development of special purpose computer programs.
7. Several programs have been written to produce administrative and accounting tabulations of the data stored on the system.

The content and flexibility of the retrieval summaries generated by the Census System have provided the Research Program with a valuable "index" to patient records. The daily routine of update and audit have eliminated the inaccuracies and incompleteness of previous disjoint and ill-kept data bases. Eight years of continuous operation of the Census System have provided BCRP with a substantial data base of information on cancer patients. Future developments will concentrate on effective utilization of the information thus obtained. These developments will be in the form of programs to correlate census data with data compiled by other data systems at the BCRP.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Project Number 201 GM 06917-07 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) CVP versus CAVP for Disseminated Non-Hodgkins's Lymphomas		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI	Richard Kaplan, M.D.	Senior Investigator COB NCI
Other	Peter H. Wiernik, M.D.	Chief, Clin.Oncol.Branch COB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) One hundred previously untreated patients with Stage III or IV <u>non-Hodgkin's lymphomas</u> were randomized to receive either <u>CVP</u> (Sytoxan = 1.5 gm/m ² IV day 1, vincristine = 1.4 mg/m ² IV days 1 and 8, prednisone = 40 mg/m ² PO days 1-10) or <u>CAVP</u> (C=1.0 gm/m ² IV day 1, adriamycin = 45 mg/m ² IV day 1, V=1.4 mg/m ² IV days 1 and 8, P = 40 mg/m ² PO days 1-10). Eighty patients have completed therapy. All patients achieving complete remission than received CCNU 100 mg/m ² PO day 1, bleomycin 20 mg/m ² IM days 1 and 8, and prednisone 40 mg/m ² PO days 1-10 (CBP) every three weeks for three courses. 17/33 (52%) CVP patients achieved CR (6/14 nodular, 9/19 diffuse) as did 25 out of 45 (55%) CAVP (12/19 nodular, 13/26 diffuse). Myelosuppression was moderate with both regimens as well as remission induction in patients with advanced non-Hodgkin's lymphomas. Analysis of remission durations is being carried out and should answer the question of the role of adriamycin in various subgroups.		

Patients who are found to have advanced non-Hodgkin's lymphomas, either Stage III or IV on the basis of extensive clinical and pathologic staging procedures are randomly assigned to receive therapy with either cyclophosphamide 1500 mg/m² IV on day 1, vincristine 1.4 mg/m² on days 1 and 8, and prednisone 40 mg/m² orally on days 1-10, or cyclophosphamide 1000 mg/m² IV on day 1, adriamycin 45 mg/m² IV on day 1, vincristine 1.4 mg/m² days 1 and 8 intravenously, and prednisone 40 mg/m² orally on days 1-10. Patients who achieve pathologically documented complete remissions then undergo consolidation therapy with CCNU, bleomycin and prednisone.

One hundred patients have undergone randomization to date with 47 patients receiving CVP therapy and 53 patients receiving CAVP therapy. Seventeen of 33 or 52% of the CVP patients have achieved complete remission, as have 25 of 45 or 55% of patients receiving CAVP. No differences in the two therapy arms in remission induction or toxicities have been observed.

Conclusions so far to be drawn from this project are: (1) the combination of Cytoxan, vincristine and prednisone is effective in inducing remission in patients with disseminated non-hodgkin's lymphomas; (2) the addition of adriamycin to CVP may be advantageous in certain subgroups. Complete analysis is underway.

Proposed Course: Project will be terminated for favorable histology patients, and those will be entered on joint Bethesda-Baltimore protocol (#MB-110). Patients with unfavorable histologies will continue to be accrued to this study, pending completion of analysis.

PUBLICATIONS:

1. Diggs, C.H.: Hodgkin's Disease and Other Lymphomas. in Hartmann, P.M. (Ed): Guide to Hematologic Disorders. New York, Grune and Stratton (in press).
2. Diggs, C.H., Wiernik, P.H. and Ostrow, S.S.: nodular Lymphoma: Prolongation of Survival by Complete Remission. Cancer Clinical Trials (In press) 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06918 07 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Streptozotocin, Bleomycin, CCNU & Adriamycin Treatment of Advanced Hodgkin's Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Richard S. Kaplan, M.D. Senior Investigator COB NCI		
Others: Peter H. Wiernik, M.D. Chief, Clinical Oncology Branch COB NCI		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: .25	PROFESSIONAL: .25	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Because of the previously reported activity of the combination of <u>streptozotocin</u> 500 mg/m ² IV days 1-5, <u>CCNU</u> 100 mg/m ² orally day 1, <u>Adriamycin</u> 45 mg/m ² IV day 1 and <u>bleomycin</u> 15 mg/m ² IM days 1 and 8 (SCAB) given at 28 day intervals in patients with previously treated Hodgkin's disease, this regimen was employed for 20 patients with stage IIIB, IVA, or IVB previously untreated Hodgkin's disease. The complete response rate in this previously untreated group was 75% (15/20). Median remission duration will be in excess of 22 months with patients still being followed from 12+ to 48+ months in remission. There have been 2 relapses of disease at 5 and 12 months. Toxicities seen with this regimen were similar to those seen in the previously treated group. Moderate myelosuppression was seen with platelets being affected more than white cells. Liver dysfunction occurred in 6 patients; 5 patients had weight loss presumably secondary to chemotherapy; bleomycin pulmonary toxicity was seen in 5 patients; and 4 patients had mild increases in their serum creatinines. SCAB appears to be effective therapy for patients with advanced, previously untreated Hodgkin's disease. This investigation has been terminated and replaced by a study of MOPP alone versus alternating MOPP and SCAB in advanced untreated Hodgkin's disease.		

This project was initiated to determine the usefulness of SCAB (streptozotocin 500 mg/m² daily for 5 days IV, CCNU 100 mg/m² PO day 1, bleomycin 15 mg/m² IM days 1 and 8, and adriamycin 45 mg/m² IV day 1) in patients with advanced stage previously untreated Hodgkin's disease. Twenty-one patients initially received SCAB, but one patient was found to have diffuse histiocytic lymphoma on pathologic review. Of the remaining 20 patients, 15 (75%) have attained a complete remission, 2 patients achieved partial remission and 3 patients failed to respond. Two patients attaining complete remission have relapsed at 5 and 12 months, with one of these patients dying at 25 months. Median remission duration will be in excess of 22 months with patients still being followed from 12+ to 48+ months in remission. This combination of agents appears to have activity equal to that of MOPP for this patient population.

Toxicities seen with this regimen were similar to those seen in the previously treated group. Moderate myelosuppression was seen with platelets being affected more than white cells. Liver dysfunction not attributable to Hodgkin's infiltration of the liver occurred in 6 patients; 5 patients had weight loss presumably secondary to chemotherapy; bleomycin pulmonary toxicity was seen in 5 patients; and 4 patients had mild increases in their serum creatinines.

MOPP chemotherapy for advanced Hodgkin's disease has been considered standard treatment for these patients for the last several years. Between 60 and 80% of patients receiving MOPP will attain complete remissions. There have been few studies to evaluate other combination chemotherapeutic regimens other than MOPP in this patient group. For this reason, and because SCAB had shown activity in a less favorable group of patients, that is, those patients who had received prior therapy, the combination of streptozotocin, CCNU, Adriamycin and bleomycin was tried in patients with untreated Hodgkin's disease. It has shown activity at least equal to that reported for MOPP, and since it has been active in patients resistant to MOPP, it is now being studied in an alternating, non-cross resistant program (MOPP/SCAB) and compared to MOPP alone.

PROPOSED COURSE: Study terminated and replaced by randomized comparison of MOPP alternating with SCAB to MOPP alone.

PUBLICATIONS:

1. Diggs, C.H., Wiernik, P.H. and Sutherland, J.C.: Treatment of Advanced Untreated Hodgkins Disease with SCAB--An Alternative to MOPP. Cancer (in press) 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 201 OM 06939 05 CUB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Methotrexate, Oncovin, Asparaginase and Dexamethasone Therapy for Adults With Acute Lymphocytic Leukemia		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Robert J. Esterhay, Jr., M.D. Senior Investigator CUB NCI		
Others: Peter H. Wiernik, M.D. Chief, Clinical Oncology Branch CUB NCI		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH baltimore, MD 21201		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 3	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to improve the remission induction rate of <u>acute lymphocytic leukemia</u> in <u>adult patients</u> utilizing <u>vincristine</u> and <u>dexamethasone</u> with intermittent moderate doses of <u>methotrexate</u> followed by <u>asparaginase</u> . A second objective is to decrease the incidence of severe infections during induction therapy by utilizing oral non-absorbable antibiotics. A third objective is to determine whether intermittent <u>high dose</u> methotrexate with <u>leucovorin rescue</u> and with vincristine and dexamethasone will prolong remission duration. The fourth objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate the clinical effectiveness with the cerebrospinal fluid methotrexate levels. To date 33 patients have been entered onto this study. Eighteen patients had no prior treatment, fifteen had prior treatment. For the no prior treatment patients, five patients are still on study and 13 are off study. Complete remissions have been achieved in 14 of the 18 (78%) patients that went through induction. For the prior treatment patients, ten of thirteen (77%) achieved complete remission. The median survival for complete remission patients for both groups is 16+ months and for all treated patients for both groups is 10+ months.		

The purpose of this project is to improve the remission induction rate of Acute Lymphocytic Leukemia (ALL) in adult patients utilizing vincristine and dexamethasone with intermittent moderate doses of methotrexate followed by asparaginase. A second objective is to decrease the incidence of severe infections during induction therapy by utilizing oral non-absorbable antibiotics. The third objective is to determine whether intermittent high dose methotrexate with leucovorin rescue and with vincristine and dexamethasone will prolong remission duration in a significant percentage of adult patients with ALL. The fourth objective is to determine the efficacy of moderate and high dose methotrexate in preventing meningeal leukemia and to correlate clinical effectiveness with the cerebrospinal fluid methotrexate levels.

Patients 15 years of age and over with a diagnosis of ALL are eligible. The induction regimen consists of methotrexate (M) 100/mg/m² on day 1, vincristine (O) 2.0 mg and asparaginase (A) 500 IU/kg on day 2, and dexamethasone (D) 6 mg/m² on days 1-10. Courses of MOAD are repeated every 10 days if possible. After complete remission is obtained, the same methotrexate and asparaginase dose and schedule (without vincristine and dexamethasone) is repeated every 10 days if possible. After complete remission is obtained, the same methotrexate and L-asparaginase dose and schedule (without vincristine and dexamethasone) is repeated every 10 days for 6 courses of consolidation therapy. This is followed by 12 monthly cytarabine courses of the same vincristine dose and methotrexate 100 mg/kg on day 1, followed by citrovorum factor for 72 hours, and the same dexamethasone dose on days 2-6. Maintenance therapy consisting of monthly vincristine, weekly methotrexate 15 mg/m² PO, daily 6-mercaptopurine 100 mg/m² PO, and dexamethasone 6 mg/m², days 1-5 of each month is then given until relapse.

To date 18 previously untreated patients with ALL (median age 31, range 15-56) have entered the study and 14 (78%) have achieved complete remission. The median duration of complete remission is 7.3 months, with a range of 1 to 42+ months and a median survival for previously untreated, complete remission patients of 16.7+ months and 10.7+ months for all previously untreated patients. Seven of 14 patients who achieved complete remission have relapsed, 2 with meningeal leukemia. Two died of infection in complete remission during consolidation and five remain in complete remission from 4+ to 42+ months. During high-dose methotrexate treatment therapeutic CSF levels of methotrexate were achieved. Ten of 13 (77%) previously treated patients (median age 31, range 15-52) also achieved complete remission. The median duration of complete remission is 7.2 months and median survival from the start of MOAD therapy is 15.7+ months for previously treated, complete remission patients and 11+ months for all previously treated patients. Four of 10 patients who achieved complete remission had marrow relapses. One patient died of a pulmonary embolus in complete remission and five remain in complete remission at 12+ and 46+ months. The major toxicity was an allergic reaction to the E. coli strain

of asparaginase, necessitating subsequent use of the Erwinia strain in over 70% of the patients. In addition, stomatitis was greater (69%) during induction with methotrexate - asparaginase rescue than during cytorreduction with methotrexate - folinic acid rescue (9%). High-dose methotrexate appears to delay the onset or reduce the incidence of meningeal leukemia compared to our historical experience with either no meningeal or pyrimethamine (Daraprim) prophylaxis. MOAD is superior to other regimens that the BCRP has used for adult ALL.

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Mycosis Fungoides Cooperative Group

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard S. Kaplan, M.D.	Senior Investigator	COB NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Dean Brenner, M.D.	Clinical Associate	COB NCI

COOPERATING UNITS (if any)
Stanford I. Lambert, M.D. Chairman, Steering Committee, Baltimore City Hospital
Baltimore, Maryland, All institutions in the Cooperative Study Group

LAB/BRANCH
Clinical Oncology Branch

SECTION:
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Baltimore, MD 21201

TOTAL MAN-YEARS: .25	PROFESSIONAL: .25	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Mycosis Fungoides Cooperative Group was founded in order to study both the natural history of and various treatment regimens for mycosis fungoides in all stages of the disease. To date, 400 cases of pathologically documented mycosis fungoides have been accrued and follow-up information is current on more than 95% of these patients. All data have been computerized and are in the process of being examined to seek correlations with various demographic, history and physical examination, laboratory and pathologic data. Two randomized studies are also in progress and have accrued 80 patients - one is for patch testing of cutaneous disease and the other is for chemotherapy of visceral disease. The Baltimore Cancer Research Program has entered four patients onto the latter study. Two patients failed therapy, one patient died from bleomycin pulmonary toxicity, and one patient cleared her peripheral blood of Sezary cells but did not clear her skin. Of the 400 study patients who are being followed, but not on chemotherapy protocols from the study group, there have been 14 from the BCRP and 9 patients continue to be followed by the BCRP. There are no firm conclusions yet from this study.

Mycosis fungoides is frequently an indolent skin disease that presents many years before any diagnostic pattern appears on pathologic specimens. The first goal of the Mycosis Fungoides Cooperative Group has been to assess what the course of mycosis fungoides is from the time of pathologic documentation as well as what the course of pre-mycosis fungoides lesions may be. The second goal is to determine optimal therapy in patients with advanced cutaneous disease and in patients with visceral mycosis fungoides.

Patients who have received previous therapy for mycosis fungoides are presently being followed on no protocol therapy by the Cooperative Group in order to determine both the natural history of the disease and to accumulate more information concerning these patients. Patients who have received no prior therapy are being randomized to one of several treatment regimens. Patients with poor prognosis cutaneous disease without visceral mycosis fungoides, that is, those patients who have plaque disease over greater than 10% of their body surface area and palpable lymphadenopathy, or those patients with multiple tumors with or without lymphadenopathy, are being randomized to receive electron beam radiotherapy or topical nitrogen mustard therapy. In addition, some patients are being randomized to receive no adjuvant systemic therapy, single-dose methotrexate therapy at 500 mg/m² followed by citrovorum rescue every 3 weeks, with dosage increment on each successive course, or to low-dose CHOP chemotherapy with cyclophosphamide 500 mg/m² IV, adriamycin 40 mg/m² IV, and vincristine 1.4 mg/m² IV plus prednisone 60 mg/m² orally days 1-5 repeated every 21 days. Those patients with visceral mycosis fungoides are allocated to one of three treatment regimens. Regimen 1 consists of methotrexate 500 mg/m² with dosage increments as described above. Regimens 2 and 3 consist of methotrexate with citrovorum rescue, adriamycin, and bleomycin either given concurrently or given on successive weeks.

Four patients have been randomized from the BCRP to receive chemotherapy. Two patients failed low-dose CHOP, one patient experienced fatal bleomycin pulmonary toxicity, and the other patient completely cleared her peripheral blood but not her erythroderma. There are an additional 8 patients who are being followed at the BCRP, having been entered only in the demographic part of this study.

Four hundred cases have been accrued for follow-up study, and 80 patients have been randomized to receive one of the therapy arms. These patients are now being analyzed by the headquarters office and the results should be available soon.

PROPOSED COURSE: Since we see so few new patients with disease stages suitable for the cooperative group studies, we have terminated our participation in the group.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09108 02 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Phase II Evaluation of PALA in Metastatic Adenocarcinoma of the Colon or Rectum		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: David A. Van Echo, M.D. Senior Investigator COB NCI Others: Peter H. Wiernik M.D. Chief, Clinical Oncology Branch COB NCI		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Once phase II study <u>PALA</u> (NSC 224131) has been used at a dose of 1500 mg/m ² IV daily for 5 days for patients with <u>metastatic adenocarcinoma</u> of the <u>colon</u> or <u>rectum</u> . Twenty-one patients have been treated to date, 6 of whom have had no prior chemotherapy, with the remainder being treated after failure of other chemotherapeutic agents. No patients has shown regression of disease enough to qualify for a partial remission. There have been two patients who have shown stabilization of disease for periods up to six months. The predominant toxicities seen with PALA have been diarrhea and erythematous skin rash which has been seen in every patients. Myelosuppression has not been a problem. PALA does not appear to have any activity in patients with metastatic carcinoma of the colon or rectum.		

Patients with advanced metastatic colorectal carcinoma have not shown dramatic responses to any known chemotherapeutic agents. 5-fluorouracil has been the agent that has been used to the greatest extent with disease responses being seen from 15 to 20% of the time. Little prolongation of survival has been seen with 5-fluorouracil alone or in combination with nitrosoureas. Because of this lack of responsiveness to various agents, phase II studies have been initiated to determine the effectiveness of other possibly useful chemotherapeutic agents.

Twenty-one patients with colorectal carcinoma have been treated with PALA at a dose of 1500 mg/m^2 intravenously days 1 through 5. Courses are repeated every three weeks, six patients had received no prior chemotherapy. No patient has shown a response to the drug, although two patients have had stabilization of previously increasing disease for periods up to six months.

Conclusion: Study closed. PALA is ineffective in colorectal carcinoma.

Proposed course: Discontinue investigation.

Publications:

1. Van Echo, D.A., Diggs, C.H., Scoltock, M., Wiernik, P.H.: Phase II evaluation of PALA in Metastatic adenocarcinoma of the colon or rectum. Cancer Treatment Reports (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09109-02 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Alternating MOPP/SCAB vs MOPP Alone for Treatment of Advanced Hodgkin's Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Peter H. Wiernik, M.D. Others: Richard Kaplan, M.D. Robert C. Young, M.D.	Chief, Clinical Oncology Branch Senior Investigator Chief, Medicine Branch	COB NCI COB NCI MB NCI
COOPERATING UNITS (if any) Medicine Branch, DCT, NCI		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Twenty-five patients with advanced previously untreated <u>Hodgkin's disease</u> have been randomized to receive either standard MOPP chemotherapy (nitrogen mustard, vincristine, procarbazine and prednisone) or standard MOPP chemotherapy alternating every other month with <u>SCAB</u> (streptozotocin 500 mg/m ² IV days 1-5, CCNU 100 mg/m ² PO day 1, adriamycin 45 mg/m ² IV day 1 and bleomycin 15 mg/m ² IM days 1 and 8). Eleven patients have been randomized to receive MOPP alone and 14 have received the combination of MOPP alternating with SCAB. No significant differences have yet emerged from the study.		

MOPP chemotherapy has become the standard treatment for patients with advanced previously untreated Hodgkin's disease. The combination of streptozotocin, CCNU, adriamycin and bleomycin (SCAB) also has shown activity equal to that of alternating two effective four-drug chemotherapeutic regimens, patients with stage III₂A, IIIB and IVA and IVB Hodgkin's disease have been randomized between standard MOPP chemotherapy and alternating every other month MOPP/SCAB.

Twenty-five patients have been randomized on this study to date, 14 receiving MOPP/SCAB, 11 receiving MOPP alone. Too few patients have completed treatment to make any assessment of the comparative effectiveness of either of these regimens.

Although MOPP chemotherapy has become standard treatment for patients with advanced, previously untreated Hodgkin's disease, there remains a sizeable patient population who will not initially respond to MOPP. SCAB also has shown activity in this same group of patients and has shown activity in previously treated patients who had received MOPP or MOPP-like regimens. Because of the activities of both of these regimens, they have been used in combination alternating them on an every-other-month basis. In order to assess whether this cyclical chemotherapy is equal to that seen with MOPP alone, a control group of patients has been treated with MOPP chemotherapy. To date, however, few patients have been randomized on either arm of the study and fewer still have completed treatment. The study is still accruing patients.

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

 Study of Two Chemotherapy Regimens for Previously Treated
 Non-Hodgkin's Lymphomas

 NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
 PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard S. Kaplan, M.D.	Senior Investigator	COB NCI
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Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
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COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH	Baltimore, MD 21201
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TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☐ (c) NEITHER
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☐ (a1) MINORS	☐ (a2) INTERVIEWS
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SUMMARY OF WORK (200 words or less - underline keywords)

Thirty-one patients with advanced previously treated non-Hodgkin's lymphomas who
 had failed to attain complete remission on combination chemotherapy or who had relapsed
 after receiving combination chemotherapy and attaining a remission have been treated with
 one of two combinations of drugs. Initially, patients who had not received an adriamycin
 containing regimen were treated with a combination of adriamycin, VM-26 and bleomycin
 and patients who had received adriamycin previously were treated with platinum and VM-26
 and bleomycin. Ten patients were treated with the platinum-containing combination, none
 of whom responded to treatment. This arm was therefore discontinued. Thereafter all
 patients were treated with the adriamycin combination regardless of previous exposure to
 the drug. Twenty-one patients have received this therapy with five patients attaining
 complete remissions and five patients partial remissions. Nine patients failed to respond and
 the remainder of the patients are still receiving treatment. Toxicities that have been seen
 have been predominantly myelosuppression. Because of its inactivity, the platinum arm has
 been discontinued while the adriamycin combination seems relatively active regardless of
 previous exposure to the drug and continues to accrue patients.

This study was initiated to study the effectiveness of two chemotherapeutic regimens for patients with previously treated advanced non-Hodgkin's lymphomas. Patients with biopsy proven recurrent or progressive non-Hodgkin's lymphomas of all histologies were initially allocated by prior adriamycin treatment to one of two regimens. For those patients who had not received adriamycin, this drug at a dose of 60 mg/m² IV day 1 plus VM-26 50 mg/m² IV day 1 and bleomycin 10 mg/m² IM days 1 and 15 were administered. For those patients who had received prior adriamycin, platinum at a dose of 50 mg/m² IV day 1 was substituted for the adriamycin with the same dosages of VM-26 and bleomycin.

In this phase of the study, 10 patients received the platinum arm. No patient responded to the platinum-containing arm and it was discontinued. The adriamycin combination arm was then opened to all patients regardless of previous exposure to adriamycin and 21 have been treated, 5 achieving a complete remission and 5 a partial remission. Nine patients have failed to respond with the remainder (2) still being under treatment. It is of interest that some patients have responded after prior exposure to single agent adriamycin or adriamycin in another combination.

Patients who fail to respond to their initial chemotherapeutic regimen fare poorly, frequently dying from progressive lymphoma. There have been few combination regimens reported for use in this patient population. Many drugs, however, have shown activity when used as single agents either for patients with previously untreated disease or for patients who have received prior chemotherapy. All of the agents used in this study have been drugs that have shown such activity. Unfortunately, no patient showed a response to platinum, VM-26 and bleomycin, so that arm has been discontinued. Because of the responses being seen with adriamycin, VM-26 and bleomycin, this arm continues to accrue patients and an ongoing analysis of response durations will ascertain the relative value of responses to adriamycin + VM-26 + bleomycin in patients that may have previously "failed" adriamycin.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09111 02 C0B
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PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Pharmacokinetic Phase I and Phase II Studies with Thymidine (NSC 21548)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Others:	Kerry Van Sloten, R.N.	Chemotherapy Nurse	COB NCI
	Daniel Zaharko, Ph.D.	Head, Pharmacokinetics and Pharmacodynamics Section	LCP NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Oncology Branch
SECTION:

Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH Bethesda, MD 21201

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
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CHECK APPROPRIATE BOX(ES)
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To investigate the toxicity and potential anti-tumor activity of Thymidine (TdR), a naturally occurring substance, twelve patients with refractory malignant disease were treated with TdR. The toxicities encountered with the dose of 75 g/m²/day TdR given as a continuous infusion for five days were tolerable and reversible. The dose limiting toxicity has been myelosuppression, especially manifested as leukopenia. Thrombocytopenia and anemia were also observed. Other toxic effects were minimal and included gastrointestinal and central nervous system. Although no significant responses were seen in this group of patients with far advanced metastatic disease, minor responses occurred in melanoma and acute leukemia. The number of patients per disease category studied so far is too small for evaluation of antitumor activity. This study is closed.

TdR, a naturally occurring nucleoside, has been shown to inhibit DNA synthesis when given in excess amount. Cytostatic activity of excess TdR against neoplastic cells has been observed both in tissue culture (Lee, S. et al, J. Cell. Physiol. 92:401-405, 1977) and in nude mice heterotransplanted with human xenografts (Lee, S. et al, Cancer Letter 3:209-214, 1977). Recently, clinical interest in TdR has also been stimulated by preclinical and clinical evidence that TdR administration can alter the toxicity and therapeutic efficacy of antimetabolites, such as methotrexate and 5-fluorouracil. This study was designed to assess the toxicity, pharmacokinetics, and anti-neoplastic activity of high dose TdR given as a single agent.

Twelve patients (pts) with advanced malignancies received 75 g/m²/day of Thymidine (TdR) given as a continuous infusion I.V. for five days. Hematologic toxicity was as follows: leukopenia (57%), thrombocytopenia (29%), and anemia (52%). Non-hematologic toxicities were gastrointestinal (nausea, vomiting and anorexia 91%, diarrhea 73%, and indigestion 22%) and central nervous system (somnia 82%, severe headaches 73%, visual illusions 56%, and memory impairment 22%). Alopecia occurred in pts. who had received more than five courses. No renal or hepatic toxicity or allergic manifestations were observed. Thymine crystals were noted in the pts. urine after refrigeration. Tumor regression (PR) occurred in one pt. with melanoma; three out of four pts. with acute leukemia had decrease in peripheral WBC counts and blasts but no bone marrow response; one pt. with ANLL also had shrinkage of hepatosplenomegaly; three pts. with colon cancer and one with unknown primary had stable disease. Pharmacokinetic studies have shown that millimolar concentrations of TdR and thymine were reached within 12 hours and maintained during the entire period of treatment. The plasma half-life of TdR was approximately 100 minutes. Forty-67% TdR and 13-18% thymine were excreted in the urine. One-third of the plasma TdR concentration was measurable in the cerebrospinal fluid.

Conclusion: TdR can safely be given by continuous infusion in large enough quantities to produce plasma concentrations of TdR and thymine in the millimolar range. This concentration has been shown in preclinical studies to produce an antitumor effect.

Because high dose TdR produces leukopenia and is known to enhance the activity of arabinosylcytosine (Ara-C) a phase II leukemia trial with Ara-C + TdR will be undertaken (see Z01 CM 06960-03--COB Project D).

This phase I study with TdR is closed.

Proposed course: Discontinue investigation

Publications:

1. Chiuten, D.F., Wiernik, P.H., Zaharko, Edwards, L.: Clinical phase I-II and pharmacokinetic study of high-dose thymidine given by continuous intravenous infusion. Cancer Research 40:818-822, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09114 02 COB
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PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Antigen-Antibody Complexes in Kidneys Postmortem

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John C. Sutherland, M.D. Research Pathologist COB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI NIH Baltimore, MD 21201

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study has been to identify antigen-antibody complexes by immunofluorescence in the kidneys of patients, especially those with leukemia and lymphoma, postmortem. The presence of such complexes may denote a chronic viral infection in which there is a minimal host response to virus resulting in the production of circulating complexes which are in antigen excess. In the early part of this decade two kidneys with antigen-antibody complexes and coming from patients with acute leukemia stained positively with antisera to the cat leukemia virus. This has not been repeated, however, in other leukemic kidneys although a kidney from a patient with SLE stained positively with cat leukemia viral antiserum. Two kidneys from Uganda stained with antiserum to EBV, one from a patient with Burkitt's lymphoma and the other from a patient with Hodgkin's disease. Further six kidneys out of 11 from American patients both with and without tumors contained Ab to VCA in their acid eluates. These findings suggest that EBV-related immune complexes may be important in diseases other than Burkitt's lymphoma.

Kidneys are collected postmortem from patients undergoing autopsy at the University of Maryland Hospital. Most of the kidneys collected are from BCRP patients. Well over 900 kidneys have been evaluated and antigen-antibody complexes found in about 10%. Many of these positivities have been further tested by staining with a battery of viral antisera which include most of the known oncogenic viruses.

Recently, I have begun to use the peroxidase, antiperoxidase (PAP) technique to stain kidneys fixed by formalin and embedded in paraffin. The leukemia viral antigen of AKR mice are readily demonstrated by this technique in the glomeruli of these mice and I plan to use this technique to extend the search for viral antigens to autopsies for which no frozen material is available and also to surgical biopsies of kidneys from patients with a wide range of kidney diseases.

The two leukemic kidneys positive for FeLV antigen were restained with anti-FeLV serum using the PAP technique and were negative. These paraffin blocks, however, were over 10 years old. The stability of the FeLV antigens in paraffin-embedded material is not know.

Because of other priorities, this project is being discontinued.

Publications: None.

Proposed Course: Discontinue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09115 02 COB
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PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Searching for Alpha Fetoprotein Ag-Ab Complexes in Placentas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: John C. Sutherland, M.D. Research Pathologist COB NCI

COOPERATING UNITS (if any)
Department of Obstetrics & Gynecology
The Johns Hopkins University

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI NIH Baltimore, MD 21201

TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to see if antibody (Ab) to AFP can be identified in human placentas including those from patients with toxemia. So far we have tentative evidence that such complexes are present in, at least, one placenta from a patient with toxemia. AFP seems to be an immunosuppressive compound and the human host appears to be tolerant to its presence both during gestation and malignancies such as carcinoma of the liver. Whether it is responsible for human tolerance to the placenta as well as to certain malignancies is not known. However, perturbations in the host response to this compound may be relevant not only to normal pregnancies but to certain tumors as well.

Whole placenta is homogenized and the homogenate washed to free the placenta of serum. The washed homogenate is then suspended in acid glycine buffer at pH 2.8. The acid eluate is then brought to 0.15 molarity and neutrality and then concentrated by ultrafiltration. Samples are then incubated with immunobeads (Biorad) coated with antihuman Ig and with antihuman albumin. The supernates are then tested for AFP by radioimmunoassay and a reduction of the level of AFP in the sample incubated with anti Ig as compared to the sample incubated with antialbumin is taken to indicate that the AFP was linked to antibody and thus precipitated by the immunobeads.

Five placentas, 4 from toxemic and 1 from normal pregnancies, were examined in this manner. Although the IgG levels were uniformly depressed, there was no depression of AFP and no AFP could be detected in acid eluates of the beads after incubation with the placental specimens. It is concluded that AFP-anti AFP complexes were not detected in acid eluates of this group of placentas. This project is discontinued at least for the present.

Publications: None.

Proposed Course: Discontinue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09116 02 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Searching for the EBV antigen in Human Tumors using the PAP technique		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: John C. Sutherland, M.D. Research Pathologist		COB NCI
Others: Paul H. Levine, M.D.		LVC NCI
COOPERATING UNITS (if any) Department of Pathology The Johns Hopkins University		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI NIH Baltimore, MD 21201		
TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Because of the ubiquity and known oncogenicity of EBV, it is appropriate to determine if EBV antigen can be identified using the PAP technique in paraffin-embedded sections of a variety of human tumors. Thus far, in working with positive controls, we have identified the VCA antigen in cultured Burkitt cells which were fixed in formalin and embedded in paraffin. Thus far we've been unable to identify the EBNA antigen in similarly processed Raji cells. We are now preparing to use a freshly available monoclonal antibody to EBV utilizing both the PAP and immunofluorescent techniques on both paraffin-embedded and frozen material.		

Burkitt cells will be fixed in acetone and others fixed in formalin. The formalin-fixed cells will be embedded in paraffin and sectioned. Both sets of cells will be stained with monoclonal mouse anti EBV using the PAP technique. Further searching for EBV antigens in human tumors will depend on the results of these studies. If only the air-dried and acetone fixed cells are positive, then further staining of human tumors, especially lymphomas, will be restricted to fresh frozen material. If the paraffin embedded cells are positive, then the search will be enlarged to stored paraffin blocks.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 401 CM 09118 02 COB
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PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Treatment of the Resistant Phase of Chronic Myelogenous Leukemia with
5-Azacytidine and VP16-213

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles A Schiffer, M.D.	Head, Cell Component Therapy Section	COB NCI
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any)
Hospital De Clinicas "Dr. Manuel Quintela", Montevideo, Uruguay

LAB/BRANCH
Clinical Oncology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION:
NCI, NIH Baltimore, MD 21201

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
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CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MILEURS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A total of 19 patients with CML in blast crisis have been treated with the combination of 5-Azacytidine (150 mg/m², IV in 3 divided doses) and VP16-213 (75 mg/m², IV per day for a total of 5 days). Prompt antileukemic effect was seen in 16 of 17 evaluable patients with one short complete response. Overall median survival was two months and response tended to be of short duration. Although the combination of 5-Azacytidine and VP16 has activity in CML in blast crisis, responses have been of short duration similar to results achieved with other agents with this disorder.

PROJECT DESCRIPTION

Z01 CM 09118 02 COB

Both 5-Azacytidine and the podophyllotoxin VP16-213 have shown some activity against resistant phase CML as single agents and were, therefore, tested in combination. Induction therapy consisted of a maximum of three 5-day courses of 5-Azacytidine (150 mg/m^2 IV in 3 divided doses) and VP16-213, 75 mg/m^2 IV/day. Nineteen patients (13m, 6F; median age 36, range 19-65) have been treated to date of whom 17 have completed therapy and are evaluable. No patient had "lymphoid" histology and terminal transferase was not present in the blasts of 8 patients tested. Prompt antileukemic effect was seen in 16/17 patients with 1 CR and 11 patients had significant cyto-reduction and hematopoietic improvement. Responses were of short duration however and the overall median survival was 2 months. Four patients are alive between 4+ and 7+ months. Myelosuppression, moderately severe nausea and vomiting, muscle aches and severe mucositis in two patients were the major side effects. Toxicities tended to decrease with subsequent courses and some patients could be treated on an outpatient basis. Although the combination of 5-Azacytidine and VP16-213 has activity in CML in blast crisis, responses have been of short duration, similar to results achieved with other agents in this refractory disorder.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09119 02 COB
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PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Search for Particle-Lamellar Complexes in Human Placentas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John C. Sutherland, M.D.	Research Pathologist	COB NCI
Others:	William G. Banfield, M.D.		CLP NCI
	Cecil W. Lee		CLP NCI

COOPERATING UNITS (if any)
Department of Obstetrics & Gynecology
University of Maryland Hospital

LAB/BRANCH
Clinical Oncology Branch
SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI NIH Baltimore, MD 21201

TOTAL MANYEARS: 6	PROFESSIONAL: 4	OTHER: 2
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CHECK APPROPRIATE BOX(ES)
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
 Two particle-lamellar complexes were noted in placental tissue from a normal pregnancy. These complexes were in a pericyte within the core of a villus. The purpose of this study was to determine how extensive these complexes were in placentas from normal and toxic placentas. Although these complexes are seen in a wide variety of tissues, they are most common in lymphoma and leukemia cells, especially hairy cell leukemia. What their purpose is and what function they play in cellular metabolism is unknown. A total of 10 placentas were examined, 7 from normal pregnancies and 3 from pregnancies complicated by toxemia. Besides the 1 normal placenta, these complexes were found in an additional placenta, one from a pregnancy complicated by postpartum toxemia.

Tissues from five placentas were obtained approximately one hour after delivery and fixed in a mixture of 1% formaldehyde and 1% glutaraldehyde buffered with phosphate. These tissues were later minced, postfixed in 1% of osmium tetroxide buffered in 0.1 M-scollidine, stained en bloc in uranyl acetate, dehydrated in graded alcohols and embedded in Epon 812. Sections cut with a diamond knife were stained with uranyl acetate and lead citrate and viewed in a Siemens Elmiskop 1A electron microscope. Six grids from 2 blocks were examined before considering a placenta negative for LPCs.

Publications: Sutherland JC, Middleton EB, Banfield WG, and Lee EH: Lamella-particle complexes in the human placenta. In Sandritter, W. (Ed.): Pathology, Research and Practice. Stuttgart, W. Germany, Gustav Fischer Verlag (in press).

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Implement a Problem-Oriented Medical Information System (PROMIS) at the BCRP

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Robert J. Esterhay, Jr., M.D. Senior Investigator COB, NCI

Other: John L. Foy, M.D., Ph.D. Medical Investigator NCHSR
Peter H. Wiernik, M.D. Chief, COB COB, NCI

COOPERATING UNITS (if any)

Intra-agency Agreement no. Y01-CM-80109
National Center for Health Services Research (NCHSR),
Office of the Assistant Secretary of Health (OASH), DHEW

LAB/BRANCH

Clinical Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MAN-YEARS:

2

PROFESSIONAL:

2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER☐ (a1) MAIL-ORG ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The BCRP, DCT has entered into an agreement with the National Center for Health Services Research (NCHSR), Office of the Assistant Secretary for Health (OASH) to develop, implement, and install a completely operational, functioning, computerized, expandable and exportable Problem-Oriented Medical Information System at the BCRP.

It is anticipated that the work should be completed in three phases, taking a total of approximately three years. Work has been completed on Phase I and Phase II has been initiated. Phase III will start upon completion of Phase II.

The BCRP, DCT has entered into an Intra-agreement with the NCHSR, OASH, DHEW, to develop, implement, and install a completely operational, functioning, computerized expandable and exportable PROMIS system at the BCRP.

The work should be completed in three phases, taking a total of approximately three years. Phases I and III should take approximately one year each to complete. Phase II would run concurrently with Phase I for the first year and will require an additional year to complete. Phase III will start upon completion of Phase II.

The NCHSR will perform the following tasks during the phases indicated:

Phase I

- A. Develop a set of system specifications for implementing the PROMIS system at BCRP in accordance with their current contract with the University of Vermont.
- B. Schedule a critical design review of the system specifications developed in A.
- C. Procure, assemble and test system hardware and software in accordance with the specifications developed.
- D. Provide site preparation consultation services to BCRP for installation of the system. Actual site preparation is the responsibility of BCRP.

Phase II

- A. Train BCRP personnel in the operation and maintenance of the system at BCRP, which will include:
 1. Development of a training plan;
 2. Training of computer personnel in hardware and software operation and maintenance;
 3. Training the users of the system (i.e., physicians, nurses, pharmacists, technicians, etc.) in the operation of the system;
 4. Prepare the necessary documentation for the training.
- B. Demonstrate that the system is operational and that it is ready for installation at BCRP.
- C. Ship and install the system at BCRP.
- D. Demonstrate to the BCRP Principal Investigator that the system is fully operational and ready for use at the BCRP.

Phase III

- A. Maintain and update the system software, keeping the tables and frames current.
- B. Test patient population study software.

This is the second year of this 3 year intra-agency agreement. One Central Processing Unit (CPU) has been procured and is being used by the Baltimore Cancer Research Program (BCRP) in Baltimore. It is located in a temporary installation site. The second CPU can be procured when the permanent installation site is available.

Tasks A, B, & D of Phase I under this intra-agency agreement have been completed. Task C has been partially fulfilled in that one CPU and associated hardware have been procured, assembled, and are currently being tested in accordance with the specifications developed. With our concurrence the second CPU and associated hardware have not been procured due to the fact that the site for the permanent installation has not been completed. It is anticipated that the site will be completed by July 1981. As specified, the tasks of Phase II have been initiated. Since there has been a significant delay in the permanent site preparation, this agreement will have to be extended beyond September 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09124-01 COB									
PERIOD COVERED October 2, 1979 through September 30, 1980											
TITLE OF PROJECT (80 characters or less) Radiotherapy, Chemotherapy or Combined Modality Treatment for Early Hodgkin's Disease											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT											
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Peter H. Wiernik, M.D.</td> <td style="width: 33%;">Chief, Clinical Oncology Branch</td> <td style="width: 33%;">COB NCI</td> </tr> <tr> <td>Others: Robert C. Young, M.D.</td> <td>Chief, Medicine Branch</td> <td>MB NCI</td> </tr> <tr> <td>Eli Glatstein, M.D.</td> <td>Chief, Radiation Oncology Branch</td> <td>ROB NCI</td> </tr> </table>			PI: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI	Others: Robert C. Young, M.D.	Chief, Medicine Branch	MB NCI	Eli Glatstein, M.D.	Chief, Radiation Oncology Branch	ROB NCI
PI: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI									
Others: Robert C. Young, M.D.	Chief, Medicine Branch	MB NCI									
Eli Glatstein, M.D.	Chief, Radiation Oncology Branch	ROB NCI									
COOPERATING UNITS (if any) Robert Slawson, M.D., Department of Radiotherapy, University of Maryland School of Medicine											
LAB/BRANCH Clinical Oncology Branch											
SECTION Office of the Chief											
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD											
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0									
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords) Eighteen patients with stages II-III _A Hodgkin's disease have been randomly allocated to receive either radiotherapy, MOPP chemotherapy, or the combination of the two modalities for treatment. The study is designed to determine the best therapy for patients with early stage Hodgkin's disease. This study is an ongoing one and represents a longterm commitment to precisely define optimal treatment for this group of young individuals with a malignant disease. In addition, the study asks whether the treatment of Hodgkin's disease might be simplified from the standpoint of expense, morbidity, and comfort by minimizing treatment for subgroups of patients who require less therapy. The longterm consequences of effective therapy will be evaluated in this study over a period of years.											

Radiotherapy has become the standard for the treatment of early stage Hodgkin's disease. However, a number of reports suggest strongly that combined modality treatment with radiotherapy and drugs results in superior disease free survival. In some studies, superior overall survival has also been noted. More recently, subgroups of patients which are more likely to benefit from combined modality treatment have been identified. MOPP chemotherapy has become the standard treatment for patients with advanced Hodgkin's disease. Pilot studies in the literature and a pilot study performed at the BCRP suggest that early stage Hodgkin's disease may be as treatable with combination chemotherapy as is late stage disease. Indeed, early results suggest that combination chemotherapy may be as effective as the standard radiotherapy approach to this disease. This study is designed to sort out the meaning of these observations. The results will be a better understanding of the treatment requirements of patients with early stage Hodgkin's disease. This will result in long term disease free survival improvement in some groups of patients. A very important question asked by this study is whether or not the treatment of Hodgkin's disease can be simplified by using only chemotherapy alone. If the results of this study support the proposition that this may be true, expenses and morbidity causing staging procedures for Hodgkin's disease could be eliminated.

An important aspect of this study is the longterm follow-up of chronic complications of each of the therapies employed. The information gained from this follow-up will be very important in determining the best overall treatment for this disease.

Eighteen patients have been randomized on this study to date. Nine have been allocated to receive combined modality treatment, 3 to chemotherapy alone, and the remainder to radiotherapy alone. It is far too early to evaluate the relative merits of these treatments. This study is still accruing patients.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT ID: Z01 CM 09136 01 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Phase II Trials in Refractory Hodgkin's Disease and non-Hodgkin's Lymphomas		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Richard S. Kaplan, M.D. Senior Investigator COB NCI		
Others: Peter H. Wiernik, M.D. Chief, Clinical Oncology Branch COB NCI		
COOPERATING UNITS (if any) Vincent Lombardi Cancer Center, Georgetown		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) new agents with a) unique modes of action or b) minimal bone marrow toxicity are being tested in relapsed and refractory lymphomas of Hodgkin's and non-Hodgkin's types with goals of better first-line combinations of agents and therapy which is safer to deliver to patients with involved bone marrow. The first drug being studied is <u>Spirogermanium</u> which has virtually no bone marrow toxicity and is unrelated to other chemotherapy agents. It is being given at 50 mg/m ² in 150 cc D ₅ W over 1/2 hour thrice-weekly for 2 weeks, then weekly. Only 2 patients have been treated thus far. The toxicity has been only mild lethargy. A second drug, the adenosine deaminase inhibitor <u>2'-Deoxycytosine</u> is also being considered for phase II studies in these diseases.		

Despite the progress of the last decade in the chemotherapy of advanced lymphomas, only a small minority of the non-Hodgkin's lymphomas can be said to be cured, and when relapse occurs, complete remission becomes more difficult to achieve and death often ensues quickly. Even in Hodgkin's disease, 50% of advanced-disease patients will ultimately fail MOPP and receive progressively less successful regimens of chemotherapy. Thus there continues to be a real need for new drug combinations which will alter these situations.

Since many second-line combinations include either drugs with mechanisms of action similar to those of the first-line drugs or several agents with overlapping bone marrow toxicity, we perceive a particular need for drugs with a) unique modes of action or b) minimal bone marrow toxicity to augment the therapeutic arsenal against lymphomas, to lead ultimately to better first-line combinations and to render safer the therapy of the patient with bone marrow involvement.

The first drug being studied is Spirogermanium, being given at 50 mg/m² (in 150 cc of D₅W over 1/2 hour IV) 3 times a week for 2 weeks, then once per week. The drug, virtually without bone marrow toxicity, is chemically unrelated to other chemotherapy drugs and has activity against lymphomas in vitro and in vivo. Thus far, only 2 patients have been entered on study and no indication of efficacy can be made. Toxicity has been only mild lethargy post-infusion.

Another agent under consideration for phase II trials is 2'-Deoxycoformycin which is selectively lymphocytotoxic and non-myelosuppressive and which acts by inhibition of adenosine deaminase. However other toxic problems with this drug remain to be worked out before it can be considered for a phase II study.

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Chemotherapy of Metastatic Genitourinary Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
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	Philip Konits, M.D.	Clinical Associate	COB NCI

COOPERATING UNITS (if any)

Urology Division, Department of Surgery, University of Maryland Hospital

LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Medical Oncology

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MAN-YEARS:

1

PROFESSIONAL:

1

OTHER:

0

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☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cis-platinum and adriamycin have been shown to have activity against testicular carcinoma. This drug combination was used for genitourinary neoplasms. Thirty-nine patients received therapy with platinum and adriamycin. Complete responses occurred in five of 11 patients partial responses occurred in four patients with testicular carcinoma. The median duration of response will exceed eight months. Partial responses have been seen in one patient with Wilms Tumor, 5/9 patients with bladder cancer, 4/9 patients with ovarian cancer and 1/4 patients with prostate cancer. Two patients are too early to evaluate, and three patients were evaluable only for toxicity. Dose reduction and delay in therapy was necessary because of bone marrow suppression. Nephrotoxicity occurred in 7 patients and necessitated discontinuing platinum one. Other toxicities included ototoxicity and neurotoxicity. The combination of platinum and adriamycin is active against advanced genitourinary neoplasms and further studies are contemplated to enhance therapeutic efficacy. Other combinations will be used for testicular cancer.

Cis-platinum and adriamycin have been shown to have activity against testicular carcinoma and other genitourinary neoplasms. Patients are given adriamycin, 60 mg/m² intravenously on Day 1 and cis-platinum 75 mg/m² intravenous infusion over 6 hour on days one and eight every three weeks. Prehydration with one liter normal saline and mannitol diuresis are also given. Doses are adjusted white count and platelet count nadirs. Thirty-nine patients with advanced genitourinary neoplasms have received therapy with platinum and Adriamycin. Complete responses have been seen in five patients. The median duration will exceed eight months. Partial response have been seen in one patient with Wilms Tumor, 5/9 patients with bladder cancer, 4/7 patients with ovarian cancer and 1/4 patients with prostate cancer. Two patients are too early to evaluate, three patients were evaluable only for toxicity. Dose reduction and creatinine 2 mg% or creatinine clearance less than 50 cc per minute) occurred in seven patients and necessitated discontinuing platinum in one patient. Other toxicities seen included ototoxicity (high frequency hearing loss and tinnitus) and neurotoxicity (peripheral neuropathy, papillidema, normal pressure hydrocephalus).

This combination of platinum and Adriamycin is active against advanced genitourinary neoplasms and further studies with additional drugs are contemplated to enhance therapeutic efficacy.

Proposed Course: Continue investigation.

Publications:

1. Ostrow, S., Hahn, D., Egorin, M., et al: Cis-dichlorodiammine platinum and Adriamycin therapy for advanced gynecological and genitourinary neoplasms. Cancer (in press).

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Initial Drug Therapy for Metastatic Renal Cell Carcinoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Van Echo, M.D.	Senior Investigator	COB NCI
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COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

Medical Oncology Section

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:

1.0

PROFESSIONAL:

0.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

AMSA has been given to 16 patients with renal cell carcinoma (RCC) without therapeutic effect and this study is closed. Chemotherapy failures are then given medroprogesterone and tamoxifen. To date, 0/10 patients have responded but this study is still in its preliminary stages and is being continued.

Renal cell carcinoma has notoriously been refractory to chemotherapy and radiation. Clinical studies in this area are attempting to identify new active chemotherapy agents or hormonal combinations.

PROJECT A AMSA

See Z01 CM 09117 -1 COB Project D.

PROJECT B Hormonal Agents

Hormonal agents, namely progestational agents, have been used in the treatment of advanced renal cell carcinoma for several years. In a recent review of the subject, the response to progestins or androgens ranged between 6 and 33 percent with an overall objective response rate of approximately 15 percent. Most responses to treatment in this tumor are, however, subjective, and this is a relatively worthless measurement of tumor response because of the diverse behavior of this tumor. In a recent clinical trial by Eastern Cooperative Oncology Group, nafoxidine (an antiestrogen) was found to have a small objective response rate in the treatment of renal cell carcinoma with two complete and one partial objective tumor responses. Another antiestrogen, tamoxifen, has shown evidence of objective response in two of four patients treated. The latter antiestrogenic compound is desirable over nafoxidine since it does not produce skin photosensitivity, but has at least a similar if not superior level of antitumor activity in human breast cancer. Several patients in a current study by the Southwest Oncology Group have had objective tumor regression.

To date, 10 patients have received the combination of medroxyprogesteron 1000 mg/wk i.M. plus tamoxifen 10 mg t.i.d. for at least eight weeks. All patients have progressed. Toxicities have included mild nausea and fluid retention (with resultant sequelae, i.e. headaches, leg swelling and mild hypertension). These effects have been easily controlled with diuretics.

Conclusion: This project is still very preliminary and will require an additional 10 patients before any valid conclusions can be made.

Proposed Course: Continue investigation.

Publications:

1. Van Echo, D.A., Markus, S., Aisner, J., Wiernik, P.H.: A Phase II trial of AMSA in patients with metastatic renal cell carcinoma. Cancer Treatment Reports (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06944-05 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Treatment of Oat Cell Carcinoma of the Lung		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch COB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Medical Oncology		
INSTITUTE AND LOCATION NIH, NCI Baltimore, MD 21021		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Oat cell carcinoma of the lung is a rapidly fatal disease requiring aggressive initial combination chemotherapy. Sequential studies at the BCRP utilize combinations of active agents (cyclophosphamide, doxorubicin, and VP 16-213 as the basic regimen; and CCNU, methotrexate, vincristine, and procarbazine as an alternating regimen). One-hundred-and-thirty evaluable patients have been entered onto the sequential studies utilizing basic combination or alternating chemotherapy. Immunotherapy was not found useful for response or survival. The overall response rate now in two sequential studies continues to be over 90% with 60% complete remission rate in limited disease. Median duration of survival was 9-1/2 months and 16 months for patients with extensive and limited disease respectively. Among patients with limited disease, there is a projected 20 to 30% long-term disease free survival. Alternating chemotherapy has not provided improvement in response rate or survival. These sequential studies demonstrate that chemotherapy alone is an effective treatment modality. Toxicity in these studies was manageable by dose modification.		

Oat cell carcinoma of the lung can be considered a widely disseminated disease at presentation and therefore requires aggressive systemic chemotherapy since all patients die of metastatic disease. The combination of cyclophosphamide, doxorubicin and VP 16-213 is highly active in this disease, and the duration of survival for patients with extensive disease is 9+ months and 15+ months for patients with limited disease. Therefore, the results from this combination chemotherapy are at least as good as any reported study today including those with projected survivals. Immunotherapy as used in the first study did not add significantly to the response rate or survival. In an attempt to improve upon response rate and duration of survival, alternating combination chemotherapy with a non-cross-resistant combination utilizing CCNU, vincristine, methotrexate and procarbazine was tested, and the alternating combination did not improve response rate or survival. Patients were stratified according to whether they have limited or disseminated disease. In addition, since there has been conflicting data regarding the role of prophylactic brain irradiation to prevent CNS metastases, there was an additional randomization in the second study to whole-brain irradiation as CNS prophylaxis to prevent CNS recurrence and/or meningeal carcinomatosis.

As of April, 1980, 93 patients have been entered onto the second oat cell study which was a prospectively randomized comparison of continuous versus alternating combination chemotherapy. The response rate for limited disease was 60% complete remission and >85 percent overall response. In extensive disease the response rate was in excess of 35% complete remission rate and over 80% overall response. Median duration of survival for the two groups is projected to be >14 and 9 months respectively. It is projected that between 20 and 30% of all limited disease patients will be long-term disease free survivors. Patients who achieved a complete remission had a significantly longer survival than patients who did not, and among limited disease patients, approximately 40% of those patients who achieved complete remission are projected to be long-term disease free survivors. At the present time there are 7 patients who are long-term survivors. Patients who achieved complete remission were randomized to receive or not receive whole-brain irradiation, and among the 15 patients who were randomized to receive whole-brain irradiation there has been no occurrence to this date of CNS metastases. Among the 14 patients randomized to not receive whole-brain irradiation, there were 5 occurrences of CNS metastases including 1 patient who died with brain metastases only (proven at necropsy) and 1 patient who died with leptomeningeal carcinomatosis only (proven at necropsy). In addition, 10 patients refused randomization or refused radiotherapy to the brain. Four of these 10 patients developed CNS metastases. The comparison is highly statistically significant. Randomization to whole-brain irradiation has been stopped and all patients who achieve complete remission receive whole-brain irradiation as prophylaxis. Toxicities were generally manageable by dosage modifications. In the second study there were 13 patients who had serious infectious episodes during periods of neutropenia and 5 patients died from these infections. There were no other drug-related deaths and most patients received all therapy as outpatients.

Proposed Course: Continue investigation.

Publications:

1. Aisner, J., Aisner, S.C., Ostrow, S., Govindan, S., Mummert, K., and Wiernik, P.H.: Meningeal carcinomatosis from small cell carcinoma of the lung: Consequence of improved survival. Acta Cytologica 23:292-296, 1979.
2. Dombernowsky, P., Sorensen, S., Aisner, J., Hansen, H.A.: Cis-diamminodichloride (II) in small cell anaplastic bronchogenic carcinoma: A phase II study. Cancer Treat. Rep. 63:543-545, 1979.
3. Mauer, L.H., Weiss, R.B., and Aisner, J.: Streptozotocin an inactive agent in small cell carcinoma. Cancer Therapeutic Trials 2:59-61, 1979.
4. Aisner, J.: Lung Cancer. In Wiernik, P.H. (Ed.): Science and Practice of Clinical Medicine, New York, Grune & Stratton, Inc., 1980 (In press).
5. Aisner, J.: Combination chemotherapy alone for the treatment of small cell carcinoma of the lung. In Wiernik, P.H. (Ed.): Controversies in Oncology, New York, John Wiley & Sons, Inc., 1980 (In press).
6. Aisner, J., Wiernik, P.H.: Complications of treatment and improved survival of patients with small cell carcinoma of the lung. In Greco, F.A., Oldham, R. (Eds.): Small Cell Carcinoma of the Lung, New York, Grune & Stratton, Inc., 1980 (In press.)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06953-04 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Chemotherapy for Regionally Advanced or Metastatic Carcinoma of the Breast		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Joseph Aisner, M.D.	Head, Section of Medical Oncology COB NCI
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	Stanley Ostrow, M.D.	Senior Investigator COB NCI
	Margaret Whitacre, R.N.	Hyperthermia Nurse COB NCI
	Philip Konits, M.D.	Clinical Associate COB NCI
COOPERATING UNITS (if any) Cancer and Leukemia Group B Oncology Program, Division of Surgery, University of Maryland Hospital		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Medical Oncology		
INSTITUTE AND LOCATION NIH, NCI Baltimore, MD 21201		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) For regionally-advanced and metastatic carcinoma (Ca) of the breast, combination chemotherapy may help to achieve local control and improved survival and is used in Project A to shrink primary masses and treat overt or micrometastases. Twenty-two pts (11 stage III, 11 stage IV) were treated with combination chemotherapy. Sixteen of 20 responders underwent surgery for local control. All 10 with inflammatory Ca had elimination of the inflammatory component. Many combinations are available for recurrent or metastatic disease, but few prospective trials compare the regimens and stratify for the prognostic variables. Project B compared 3 regimens for response rates, duration of survival, and the effect of MER immunotherapy. A group effort was necessary to stratify for the variables. With 380 pts entered, prior postoperative adjuvant radiotherapy and immunotherapy were shown to be detrimental factors for response and survival. Chemotherapy containing doxorubicin is shown to be superior. New drug combinations are needed to treat pts with tumors refractory to standard drugs. Projects C and D are two new combinations of mitomycin-C and vinblastine, and doxorubicin and VP 16 respectively. With 29 and 24 pts entered, both are highly active combinations.		

Since the introduction of the five-drug combination chemotherapy program for breast cancer, there has been a large number of studies utilizing permutations of the drugs and their scheduling. In addition, the anthracycline antibiotic doxorubicin has been used in several regimens in an attempt to improve response rate, duration or survival. The active combinations have also been applied to earlier breast cancer as a postoperative adjuvant with demonstrated improvement in disease-free interval and survival. Project A utilizes combination chemotherapy for earlier breast cancer (stage III unresectable) including inflammatory carcinoma in an attempt to determine if this approach would shrink the masses allowing for a surgical procedure and primary closure. In advanced, metastatic or hormone-refractory breast cancer, the attempt has been to prospectively compare combination chemotherapy regimens which have demonstrated activity for the treatment of metastatic disease. Few previous studies prospectively compared the various treatment regimens available. Since metastatic carcinoma of the breast is a very heterogeneous disease with many factors influencing the course of the disease and the response to chemotherapy, it is necessary to do a prospective comparative trial of the various chemotherapy regimens. In order to accomplish this, a large cooperative group effort is necessary to stratify for these prognostic factors. Project B randomly compared three combination chemotherapy regimens, each of which has been shown to be an effective treatment program. In addition, this study evaluated the role of immunotherapy utilizing MER intradermal injections for its potential to increase response rate, duration and survival and alleviate drug toxicity. For patients with advanced refractory breast cancer, new agents and new combinations of agents are being evaluated in order to identify second-line chemotherapy regimens which might be utilized effectively for patients failing primary combination therapy. In addition, the identification of such non-cross-resistant combinations offers the potential for earlier treatment with these combinations or the planning of alternating combination chemotherapy regimens. Project C utilizes doxorubicin and VP 16 for patients failing primary drug treatment which did not include doxorubicin. Project D for patients who have received primary combinations including doxorubicin, a combination of vinblastine and mitomycin-C is being tested. In addition, patients who fail these combinations are placed on phase II studies and phase I studies which are reported separately.

Project A

Local, regional breast carcinoma. As of April, 1980, 22 patients (11 stage III, 11 stage IV) were treated initially with combination chemotherapy to shrink primary masses and control overt or micrometastases. Five stage III patients and 5 stage IV patients had inflammatory carcinoma. After a median of two courses, 16 of 20 responders underwent surgical procedures: 4 had radical mastectomy, 9 had modified radical mastectomy and 3 had simple mastectomy. Twelve of the 16 had primary closure without skin grafts. All 10 patients with inflammatory carcinoma had elimination of the inflammatory component. Seven of 10 had mastectomy and 2 are about to undergo surgery. For all pts local control was achieved for a median of 10+ months (range 2+ to 38). Aggressive combination chemotherapy can eliminate inflammatory Ca and reduce the size of breast masses, allowing for lesser surgical procedures, frequently with primary closure. Continuation of chemotherapy may control overt or micrometastatic disease.

Proposed Course: Continue investigation.

Project B

Primary chemotherapy for advanced metastatic disease. As of April, 1980, 382 patients have been entered onto protocol from multiple institutions. The 382 patients have been roughly equally distributed among the various treatment groups. As of the last group analysis, there were 338 evaluable patients, the remainder being either inevaluable or accrued since the last cut-off. Analysis of evaluable patients revealed that those patients who received prior hormonal therapy had a higher response rate to chemotherapy ($p=.02$) than patients who did not receive hormonal therapy. In addition, those patients who received postoperative adjuvant radiotherapy had a significantly inferior response to chemotherapy (especially for the doxorubicin-containing regimens) than the patients who received no postoperative radiotherapy. Patients who received MER immunotherapy had a significantly inferior response to chemotherapy ($p=.003$) to that of patients who received no MER. In addition, patients who received MER immunotherapy had a significantly shorter survival. Therefore, further randomization to MER immunotherapy was discontinued and all MER treatment was stopped. Analysis of the treatment arms revealed differences between the doxorubicin-containing regimens and the methotrexate-containing combination. The goals of the study are thus complete and the study will be replaced with a successor group study.

Proposed Course: Continue investigation.

Project C

Treatment of advanced refractory breast carcinoma. As of April, 1980, 24 patients previously treated and refractory to combination chemotherapy without doxorubicin were treated with a combination of doxorubicin plus VP 16-213. Nine of 24 patients achieved a response to combination chemotherapy, 3 complete responses and 6 partial responses. Doxorubicin plus VP 16 is therefore an active combination in advanced breast cancer which is non-cross-resistant with conventional chemotherapy. It is, however, myelotoxic in this previously treated group.

Project D

As of April, 1980, 29 patients have been treated with the combination of vinblastine and mitomycin-C. Ten of the 24 evaluable patients (5 are too early) showed objective tumor regression with one complete response and 9 partial responses. This combination is, however, myelotoxic in these previously heavily treated patients. Individual phase II agents and phase I agents are treated in a separate report.

Proposed Course: Continue investigation.

1. Aisner, J.: Breast Cancer. In Wiernik, P.H. (Ed.): The Science and Practice of Clinical Medicine, New York, Grune & Stratton, Inc., 1980 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06954-04 COB
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PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Chemotherapy for Carcinoma of the Head and Neck

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

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LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Medical Oncology

INSTITUTE AND LOCATION

NIH, NCI Baltimore, MD 21201

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Advanced squamous cell carcinoma of the head and neck or esophagus have a very poor five-year survival despite aggressive local treatment with surgery, radiotherapy or both. Many such patients die of metastatic disease. Therefore, a pilot trial of aggressive combination chemotherapy was warranted to determine if an improved response rate or survival can be obtained. Bleomycin, cis-platinum II with hydration, and high-dose methotrexate with citrovorum rescue are agents with activity in these tumors. Thirty-three patients were treated with combinations of these agents. Twenty-six received a three-drug combination and 7 patients received only DDP and bleomycin. There was a 73% response rate in previously untreated patients with 4 complete and 12 partial responses, and a 55% response rate in previously treated patients, all PR's. Duration of response to a single course of chemotherapy is 8-12 weeks. Toxicity included vomiting, alopecia, leukopenia, thrombocytopenia, mucositis and elevated serum creatinine. There were 2 drug-related deaths. Among the 22 responders, 12 had surgery, 5 had subsequent radiotherapy. Among the 11 failures to therapy, 3 had surgery and 1 had radiotherapy. Twelve patients received no local therapy. Similar studies are now underway in cooperative groups.

1221

Advanced squamous cell carcinoma of the head and neck have a very poor survival rate despite extensive surgery or aggressive radiotherapy or both, and many of these patients develop inanition. Some of the patients develop metastases despite control of the local tumor. Several single agents have been identified with activity in these tumors, and combination chemotherapy may thus be warranted. The current pilot study uses combinations of three active agents: bleomycin, cis-diamminedichloroplatinum (DDP) with hydration, and methotrexate with folinic acid reversal, to test the toxicity of such a combination program and to determine if such combination therapy might increase response rate or duration or make the tumors more technically resectable.

As of April 1980, 33 patients with stage III and IV (M-0) squamous cell carcinoma of the head and neck were treated with a single course of combination chemotherapy. Twenty-six received the three-drug combination and 7 received DDP and bleomycin but not methotrexate because of elevated creatinine. The response rate was 73% in previously untreated patients (16/22) with 4 complete remissions and 12 partial responses. The response was 55% in the previously treated group (6/11) all of which were partial responses. The duration of response to such a single course of chemotherapy was estimated to be 8-12 weeks. The toxicity in the 33 patients included vomiting, alopecia, leukopenia, thrombocytopenia, mucositis, and elevation of serum creatinine. There were 2 drug-related deaths, one from nephrotoxicity and one from methotrexate bone marrow suppression. In general, toxicities were manageable by careful hydration and careful folinic acid reversal. Subsequent local therapy was applied to 21 patients. Twelve of the responders had surgery and 3 of the non-responders had surgery. Five of the responders and one of the non-responders had radiotherapy. Twelve patients had no subsequent local treatments. These data are sufficiently encouraging for larger groups to undertake and similar studies are now in progress in multiple institutions.

Proposed Course: Discontinue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06955-04 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Adjuvant Chemotherapy and Combination Chemoimmunotherapy in the Treatment of Advanced Malignant Melanoma		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
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COOPERATING UNITS (if any) Department of Surgery, University of Maryland Hospital		
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SECTION Section of Medical Oncology		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) There are only small numbers of patients in all arms of the study but the results are similar to those obtained in larger groups. In the patients receiving DTIC plus MER there is approximately a 29% partial response rate with responses seen mainly in patients with disease confined to the skin. In the group receiving DTIC alone there is a 25% partial response rate again involving intracutaneous metastases. There is still no evidence that adjuvant chemotherapy delays the time to progressive disease. Accrual has been slow on this study and because large cooperative cancer groups have begun to address this question this study is closed.		

PROJECT DESCRIPTION

Z01 CM 06955 04 COB

There have been 29 evaluable patients entered on study. In the adjuvant arm of the protocol seven patients received DTIC alone and eight patients have been randomized to receive no therapy. The median survival from the date of randomization is 343+ days in the no treatment group and 288 in the DTIC group. This is not statistically significant. Several large cooperative groups including the World Health Organization with hundreds of patients have shown no benefit for melanoma patients given DTIC as adjuvant therapy. Therefore the adjuvant arm of this study is closed. In the group of patients of this study is closed. In the group of patients with advanced and measurable disease ten patients received both DTIC and MER and the median survival is 80 days. There is no statistical difference in the two groups. Because in the advanced group there is no difference in survival and time to recurrence in the DTIC vs DTIC plus MER arms of the study; the toxicities of MER and because the data is similar to larger studies, the advanced disease part of this project is closed.

Proposed Course: Discontinue investigation.

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Nitrous Oxide in the Treatment of Refractory Adult Leukemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David A. Van Echo, M.D. Senior Investigator COB NCI

Others: Peter n. Wiernik, M.D. Chief, Clinical Oncology Branch COB NCI

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Medical Oncology Branch

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:

2

PROFESSIONAL:

2

OTHER:

0

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☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Prolonged inhalation of nitrous oxide for four or more days has been shown to lead to marrow aplasia. There are scattered reports of decrease in peripheral white counts in leukemic patient, however, there have not been any systematic clinical trials. This protocol is a Phase I evaluation of nitrous oxide in the treatment of adults no longer responsive to conventional anti-leukemic therapy. The objectives of this study are to determine the role of nitrous oxide in suppressing myelopoiesis and to determine the most efficacious way to administer this therapeutic modality.

Five patients have thus far been entered onto this study receiving six separate treatments which will be evaluated. There were no effects in peripheral white count or bone marrow cellularity in any patient. Future plans are to use continuous exposures of nitrous oxide for up to 96 hours.

Prolonged inhalation of nitrous oxide for four or more days has been shown to lead to marrow aplasia. There are scattered reports of decrease in peripheral white counts in leukemic patients, however there have not been any systematic clinical trials. This protocol is a Phase I evaluation of nitrous oxide in the treatment of adults no longer responsive to conventional anti-leukemic therapy. The objectives of this study are to determine the role of nitrous oxide in suppressing myelopoiesis and to determine the most efficacious way to administer this therapeutic modality.

Five patients have thus far been entered onto this study receiving six separate treatments which will be evaluated. The first patient received concentrations of up to 40% nitrous oxide in intermittent exposures for up to 2 1/2 hours on 3 consecutive days. The second patient received intermittent inhalations of up to 40% nitrous oxide for intermittent exposures up to 12 hours on 3 successive days. The fifth patient received up to 40% nitrous oxide essentially uninterrupted for 24 hours. All patients experienced tolerable nausea and vomiting that resolved with decreasing the concentration of nitrous oxide. The sixth patient, who had a previous history of peptic ulcer disease, experienced minor hematemesis which resolved promptly with termination of nitrous oxide and administration of anti-acids. No patient experienced changes in vital signs, arterial blood gases, renal function or hepatic function. There were no effects in peripheral white counts or bone marrow cellularity in any patient. Future plans are to use continuous exposures of nitrous oxide for up to 96 hours.

Proposed course: Continue investigation

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06960 04 COB												
PERIOD COVERED October 1, 1979 through September 30, 1980														
TITLE OF PROJECT (80 characters or less) New Drug Therapy of Previously Treated Adult Leukemia														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width:100%; border: none;"> <tr> <td style="width:33%;">PI: David A Van Echo, M.D.</td> <td style="width:33%;">Senior Investigator</td> <td style="width:33%;">COB NCI</td> </tr> <tr> <td>Others: Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td>COB NCI</td> </tr> <tr> <td>Joseph Aisner, M.D.</td> <td>Head, Section Medical Oncology</td> <td>COB NCI</td> </tr> <tr> <td>Susan Markus, R.N.</td> <td>Chemotherapy Nurse</td> <td>COB NCI</td> </tr> </table>			PI: David A Van Echo, M.D.	Senior Investigator	COB NCI	Others: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB NCI	Susan Markus, R.N.	Chemotherapy Nurse	COB NCI
PI: David A Van Echo, M.D.	Senior Investigator	COB NCI												
Others: Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI												
Joseph Aisner, M.D.	Head, Section Medical Oncology	COB NCI												
Susan Markus, R.N.	Chemotherapy Nurse	COB NCI												
COOPERATING UNITS (if any) None														
LAB/BRANCH Clinical Oncology Branch														
SECTION Medical Oncology Branch														
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201														
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0												
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline key)														
Clinical Studies have been undertaken to identify new agents or combinations of agents that may have activity in patients with relapsed acute leukemia. In 12 patients the combination of <u>5-Azacytidine plus pyrazofurin</u> and in 20 patients the single agent <u>VP16-213</u> in high doses have both been found to be ineffective. <u>AMSA</u> used in 17 patients as a single dose and used in 16 patients on a multiple dose schedule and the combination of <u>cytosine-arabinoside (ARA-C) plus thymidine (TdR)</u> used in 28 patients both have shown significant antileukemic activity in patients with relapsed ALL and ANLL. The response rates (CR + PR) in either one of the last two studies is in the range of 30-50%. These studies are still ongoing and it is still too early to develop stable response data.														

Relapse from complete remission occurs in almost all adult patients with leukemia and patients become refractory to 1⁰ induction therapy. New agents are necessary to produce response and survival. Patients who relapse post initial therapy are eligible. Most relapsed patients are then treated with newer agents, however, responses are invariably infrequent and of short duration. Relapsed leukemic patients who are refractory to their initial therapies or whose initial response is very brief (or non existent) are eligible for these studies.

PROJECT A High Dose VP 16-213

VP 16-213 was given at maximally tolerated doses ($250-300 \text{ mg/m}^2/\text{d} \times 5$) to 12 acute and nonlymphocytic leukemia patients (ANLL) and one acute lymphocytic leukemia (ALL). One patient with ANLL achieved a partial response for one month. Toxicities were significant and included myelosuppression, mucositis, esophagitis and transient rises in liver function tests.

Conclusions: VP 16-213 given at 2-3 times the usual dose over 5 days does not have a higher response rate than when given at conventional levels but does have significantly more mucosal toxicity. Study closed.

Proposed course: Close project.

PROJECT B 5-Azacytidine (AZA) plus Pyrazofurin (PF)

Twenty adult patients with ANLL received the combination of AZA $150-250 \text{ mg/m}^2/\text{d}$ in three divided doses for five days plus PF $7.5-30 \text{ mg/m}^2 \times 1$ on day one. Four patients died early deaths secondary to infection or hemorrhage. Three patients achieved a CR or PR lasting 41-94 days. Hematologic toxicity was comparable to that from AZA alone at the dose and schedule used in this study. Non-hematologic toxicity was increased with the combination and included dose-limiting skin rash and mucositis felt to be secondary to the addition of PF.

Conclusion: The addition of PF to AZA produces significantly more non-hematologic toxicity than AZA alone and no therapeutic advantage over treatment with AZA alone.

Proposed course: Study closed.

PROJECT C AMSA

AMSA an acridine derivative has been given by two schedules to patients with relapsed leukemia. The first was a single dose two hour infusion at doses from $150-600 \text{ mg/m}^2$. Doses were repeated every 1-3 weeks as the bone marrow regenerated. Eleven patients with ANLL, four with ALL, one with CLL and one with CML in blast phase were entered on study. No meaningful and leukemic responses were seen although all patients had a dramatic peripheral antileukemic effect and the majority experienced transient bone

marrow hypoplasia. Phlebitis was a major impediment to employing AMSA at higher doses. Secondly 16 patients subsequently received AMSA as a one hour infusion at 60 mg/m² daily until bone marrow hypoplasia (usually 8-12 days). These responses have been three CR's in nine ANLL patients, one PR in three ALL patients and 0/4 CML blast phase patients. No patient has relapsed to date and the median duration of response will be in excess of six months. Toxicities are significant and include prolonged myelosuppression, mucositis and transient liver function abnormalities.

Conclusions: AMSA as a single dose appears to have limited activity in relapsed leukemia. When given as a multiple dose schedule AMSA demonstrates an encouraging level of antileukemic activity which deserves further study.

Proposed Course: Continue investigation

PROJECT D Ara-C plus TdR

Ara-C plus TdR was given to 28 patients with relapsed leukemia 90% of whom had received extensive prior therapy with and were resistant to Ara-C. The initial aspect of the study involved a dose finding scheme to determine a) the toxicity of the combination and b) the maximally tolerated doses that could be given. So far 250 mg/m²/d of Ara-C plus 6 gm/m²/d of TdR are reasonable and safe doses to give. Both drugs are administered simultaneously as continuous intravenous infusions daily until bone marrow hypoplasia is achieved (usually 8-12 days). Responses have been seen in 8/17 evaluable ANLL patients, 0/4 evaluable ALL patients and 0/3 evaluable CML blast phase patients. Three patients are too early to evaluate. Durations of unmaintained responses have been short (2-4 months). Patients currently achieving remission status receive two additional five day courses of Ara-C + TdR at 1-2 month intervals. It is still too early to determine if this additional maneuver will prolong remission. Toxicities have included mild rash, prolonged myelosuppression, liver function abnormalities and occasionally severe enteritis.

Conclusions: The combination of Ara-C + TdR is a highly active combination for relapsed ANLL producing acceptable toxicity. Response data for other forms of leukemia are still incomplete.

Proposed course: Continue investigation

PROJECT E Nitrous Oxide

Followed as a separate project. See Z01 CM 06957 03 COB

Publications:

1. Van Echo, D.A., Wiernik, P.H., Aisner, J.: High dose V_{10-21} (Z01 141540) for the treatment of patients with previously treated acute leukemia. Cancer Clinical Trials, (in press), 1980.

PROJECT DESCRIPTION

Z01 CM 06960 04 COB

2. Van Echo, D.A., Chiuten, D.F., Markus, S., Wiernik, P.H.: A clinical trial of pyrazofurin in combination with 5-azacytidine in acute non-lymphocytic leukemia. Cancer Clinical Trials, (in press), 1980.

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cis-dichlorodiammine Platinum II Neurotoxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Stanley Ostrow, M.D.	Medical Investigator	COB NCI
Others:	Peter W. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Merrill Egorin, M.D.	Scientific Expert	LCB NCI
	Andre Lekoy, Ph.D.	Biomedical Engineer	R BEI

COOPERATING UNITS (if any)

Department of Neurology, University of Maryland Hospital
Department of Rehabilitation Medicine, University of Maryland Hospital

LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Medical Oncology

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MIMIC ☐ (a2) INTERVIEW

SUMMARY OF WORK (200 words or less - underline keywords)

Neuropathological, biochemical and electrophysiologic studies were done with patients receiving cis-platinum. Serial nerve conduction studies on ten patients revealed evidence of an axonal type of polyneuropathy in three of the patients. Sural nerve biopsy performed on two patients revealed patterns of axonal degeneration by hematoxylin and eosin staining and by electron microscopy studies. Attempts are being made to localize platinum in nerves by electron microscopy techniques.

Cis-dichlorodiammineplatinum II (DDP) an antitumor agent possesses antitumor activity in genitourinary neoplasms, sarcomas, lymphomas and breast cancer. There have been reports of neurologic toxicity following DDP therapy being primarily peripheral neuropathy. In patients treated with platinum at doses of 50 to 100 mg/m² every three weeks alone or with combination chemotherapeutic agents we have observed sensorimotor polyneuropathies. In some cases, neurologic toxicity has necessitated discontinuance of platinum therapy and cessation of therapy has resulted in improvement in neurologic symptoms.

With cooperation of the Neurology Department at the University of Maryland School of Medicine and the Department of Rehabilitation Medicine, University of Maryland. Neuropathological, biochemical and electrophysiologic studies have been undertaken on nerves of patients who have received cis-platinum. Serial nerve conduction studies, prior to and during platinum therapy been performed on 10 patients. In three patients there has been evidence of a sensory polyneuropathy of axonal type following DDP therapy. Sural nerve biopsy performed on two patients has revealed patterns of axonal degeneration by hematoxylin and eosin stain and electron microscopy studies. However electron microscopy probe studies and x-ray diffraction analysis have failed to reveal localization of platinum in the nerves. Continued studies on patients including nerve conduction tests and further biochemical and histologic studies on nerve biopsies are planned on patients receiving platinum in combination with other neurotoxic drugs such as vindesine.

Proposed Course: Continue investigation

Publications:

1. Keinstein, L., Ostrow, S., Wiernik, P.H.: Serial nerve conduction studies in Cis-platinum (II) DDP peripheral neuropathy. Arch. Phys. Med. Rehabil. 59:558, 1978.
2. Ostrow, S., Hahn, D., Wiernik, P.H., Richards, R.D.: Ophthalmologic toxicity after cis-dichlorodiammine platinum (II) therapy. Cancer Treat Rep 62:1591-1594, 1978.
3. Keinstein, L., Ostrow, S., Wiernik, P.H.: Cis-platinum neuropathy. Arch. Phys. Med. Rehabil. 1980 (in press).

PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Phase I Studies of New Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Van Echo, M.D.	Senior Investigator	COB NCI
Others:	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
	Joseph Aisner, M.D.	Head, Section Medical Oncology	COB NCI
	Margaret Whitacre, R.N.	Chemotherapy Nurse	COB NCI
	Nicholas Bachur, M.D., Ph.D.	Chief, Laboratory of Clinical Biochemistry	LCB NCI
	Merril Egorin, M.D.	Expert, Clinical Biochemistry	LCB NCI

COOPERATING UNITS (if any)

Laboratory of Clinical Biochemistry, BCRP, NCI

LAB/BRANCH

Clinical Oncology Branch

SECTION

Medical Oncology Section

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Phase I studies are pharmacology and toxicity trials in humans of new anticancer agents that have completed preclinical toxicity, pharmacology and anti-tumor activity. Such studies are completed when dose-limiting toxicity is encountered. A study has been completed for pentamethylmelamine (NSC-118742) which showed dose limited toxicity of studies are in progress for Dihydroxy anthracenedione (NSC 301739) and Aclacinomycin-A (NSC 208734) and dose limited toxicity has not yet been reached.

Phase I studies are studies in humans of the pharmacology and toxicity of new anti-cancer agents that have already had preclinical toxicity, pharmacology and anti-tumor activity testing completed. These studies usually require 20-30 patients with refractory malignant disease. Initial dosages are based on toxicity data from laboratory animals. Dosages are escalated according to standard schemes. Three patients are treated at each non-toxic dose level and six patients at each level with reversible acceptable toxicity. When dose limiting toxicity is encountered the study is closed.

PROJECT A

Pentamethylmelamine (PMM) is a water soluble analogue of hexamethylmelamine (HMM) which is suitable for intravenous administration. HMM can only be given orally but its rate of absorption is often erratic. In addition its dose limiting toxicity is gastrointestinal. Thirty-one patients were treated with PMM using two schedules. The first was a two hour infusion once every three weeks. Dose-limiting toxicity was nausea-vomiting, anorexia and CNS-depression. the recommended dose for Phase II trials using this schedule is $1,500 \text{ mg/m}^2$ every 3 weeks. A second schedule was investigated giving PMM every other day x3 days. Again, dose limiting toxicity was nausea-vomiting and anorexia. The recommended dose for Phase II trials for this schedule is $1,000 \text{ mg/m}^2$ given every other day x3 (M-W-F) with courses repeated every three weeks. No antitumor responses were seen.

Proposed course: Close phase I investigation of this agent.

PROJECT B

Dihydroxyanthracenedione (DHAD) is an anthraquinone compound which has been investigated because of its structural relationships to the anthracyclines. It has DNA intercalating properties and preclinical antitumor activity equal to or superior to Adriamycin. In addition, since DHAD lacks a sugar moiety it is theorized that it will have less clinical cardiac toxicity which is the major limitation to the use of the anthracyclines. Ten patients have been treated with DHAD using a single dose intravenous bolus every day x3 schedule. Courses are repeated every three weeks. The only toxic side-effect observed has been reversible dose related leukopenia. Patients are currently receiving 5 mg/m^2 every day x3. It is felt the maximally tolerated dose will be in the range of 4-5 mg/m^2 every day x3. A minor tumor response has been seen in a patient with soft tissue sarcoma.

Proposed course: Continue current Phase I investigation.

PROJECT C

Aclacinomycin-A (ACM-A) is an anthracycline analogue currently in clinical trial in Japan. It appears to have similar preclinical and clinical spectrum of antitumor activity as Adriamycin. It is reported to have less nonhematologic side-effects (hair loss, nausea-vomiting) than Adriamycin and is felt to have less cardiac toxicity. Only two patients have received ACM-A on a single dose intravenous bolus schedule every three weeks. No toxicity has been observed. Detailed plasma and urine pharmacokinetics of ACM-A and its metabolites are being studied along with sequential cardiac studies to define any possible alteration in cardiac dynamics related to ACM-A use.

Proposed course: Continue investigation

Publications:

1. Van Echo, D.A., Chiuten, D.F., Whitacre, M., Aisner, J., Lichtenfeld, J.L., Wiernik, P.H.: A phase trial of pentamethylmelamine (NSC 118742, PMM) in patients with previously treated malignancies. Cancer Treatment Reports, (in press), 1980.
2. Chiuten, D.F., Wiernik, P.H., Zaharko, D.S., Edwards, L.: Clinical Phase I-II and pharmacokinetic study of high-dose thymidine given by continuous intravenous infusion. Cancer Res 40:818-822, 1980.
3. Erlichman, C., Strong, J.M., Wiernik, P.H., Edwards, L.S., Cohen, M.H., Levine, A.S., Hubbard, S.M., and Chabner, B.A.: Phase I trial of N-phosphoroacetyl-L-aspartate (PALA). Cancer Res 39:3995-3995, 1979.
4. Zaharko, D.S., Bolten, B.J., Chiuten, D., Wiernik, P.H.: Pharmacokinetic studies during Phase I trials of high dose thymidine infusions. Cancer Res 39:4777-4781, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NO. Z01 CM 00974 03 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Studies in Whole Body Hyperthermia (WBH)		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: David A. Van Echo, M.D. Others: Peter H. Wiernik, M.D. Joseph Aisner, M.D. Nicholas Bachur, M.D., Ph.D. Stanley Ostrow, M.D. Philip Konits, M.D. Margaret Whitacre, R.N.	Senior Investigator Chief, Clinical Oncology Branch Head, Section Medical Oncology Chief, Laboratory of Clinical Biochemistry Investigator Clinical Associate Chemotherapy Nurse	COB NCI COB NCI COB NCI LCB NCI COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) Laboratory of Clinical Biochemistry, BCRP, NCI		
LAB/BRANCH Clinical Oncology Branch		
SECTION Medical Oncology Section		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER:
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A method has been developed utilizing equipment provided by the National Aeronautics and Space Administration to induce <u>whole body hyperthermia</u> to 41.°C with minor morbidity to patients. Studies are currently in progress to assess the <u>pharmacokinetics</u> of cyclophosphamide (CTX) plus WBH; CTX plus Adriamycin plus WBH and the therapeutic effectiveness of these combinations in adenocarcinoma of the lung (CTX & WBH) and soft-tissue sarcomas (CTX-ADM-WBH). These studies are still in their preliminary stages and no conclusions are possible at this time.		

Clinical whole body hyperthermia (WBH) has been examined to assess the feasibility of combining cyclophosphamide (CTX) with WBH 41.8°C for four hours in patients with adenocarcinoma of the lung. In addition, seven patients with soft-tissue sarcoma have received the combination of Adriamycin (ADR) + CTX and WBH 41.8°C for two hours. Other aspects of the project include pharmacokinetic studies of the chemotherapeutic agents during WBH, and studies of endocrine function and physiologic alterations during WBH.

PROJECT A CTX + WBH

Six patients with metastatic adenocarcinoma of the lung have been given CTX 1.5 mg/m² as a two hour infusion during WBH 41.8°C for four hours 2/5 patients evaluable for response have objective tumor regressions lasting 2-7 months. Toxicities have included myelosuppression secondary to CTX in addition to the usual symptoms from WBH. Pharmacokinetics are being done on CTX. Patients serve as their own controls and receive at least one course of CTX without WBH in order to compare the results with CTX-WBH courses.

PROJECT B ADR-CTX + WBH

Seven patients with soft-tissue sarcomas have received the combination of ADR 45 mg/m² during WBH 41.8°C for two hours followed by CTX 1.0 gm/m² as a one hour infusion four hours following WBH. 1/5 evaluable patients has achieved a pathologically documented complete response. Four patients have progressed. Preliminary pharmacokinetic studies on CTX indicate that unchanged (inactive) parent compound CTX may be excreted at an accelerated rate through the urine suggesting that WBH inhibits hepatic microsomal activation of CTX. Studies of ADR pharmacokinetics are too preliminary for comment.

CONCLUSION:

WBH can safely be combined with a variety of chemotherapeutic agents in an effort to augment antitumor activity. It is essential that pharmacokinetic studies be integrated as part of any WBH program since WBH may adversely affect drug metabolism.

This project is open for further investigation and in the future will investigate the pharmacology of other anti-cancer agents when combined with WBH.

Proposed course: Continue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CM 06975-03 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Treatment of Non-Oat Cell Carcinoma of the Lung		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Joseph Aisner, M.D. Others: Clarence L. Fortner, M.S. Stanley Ostrow, M.D. David A. Van Echo, M.D.	Head, Section of Medical Oncology Head, Clinical Research Pharmacy Section Senior Investigator Senior Investigator	COB NCI COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) Department of Oncology, Health Sciences Centre, Winnipeg, Manitoba, Canada Department of Medicine, Loch Raven Veterans Administration Hospital, Baltimore Department of Oncology, Sinai Hospital of Baltimore		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Medical Oncology		
INSTITUTE AND LOCATION NIH, NCI Baltimore, MD 21201		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) A large proportion of patients with <u>non-oat cell lung cancer</u> present with surgically unresectable disease and disease outside radiotherapy ports. Many single drugs have been evaluated in the treatment of non-small cell lung cancer, but they have been disappointing. Combination chemotherapy utilizing possibly synergistic drugs may warrant testing in this disease. Since there is a suggestion that the subtypes of bronchogenic carcinoma may be related, the current study was instituted using cyclophosphamide, doxorubicin, VP 16-213 (this combination has been extremely active in oat cell carcinoma--see report #06944) with and without added platinum. This study is undertaken to determine if this combination chemotherapy can augment response and survival and to determine the additive value of platinum in this combination. Twenty patients have been entered onto the study, 9 received 3 drugs and 11 received 4 drugs. There have been 1 complete remission and 3 partial remissions. It is too early to evaluate by treatment or tumor subtype. Toxicities were manageable and included myelosuppression, mucositis and pharyngitis.		

Advanced non-oat-cell carcinoma of the lung with disease outside the realm of surgery and radiotherapy presents a significant problem, and thus the testing of combination chemotherapy programs in this disease is warranted. Cyclophosphamide and doxorubicin are single agents of known low order of activity, and there is animal data to suggest that they may be synergistic. This study builds upon the observation in patients who have mixed oat cell and non-oat-cell lung cancers that these tumors may be responsive to cyclophosphamide, doxorubicin and VP 16. This study utilizes cyclophosphamide, doxorubicin, VP 16 plus-or-minus platinum. Cyclophosphamide, doxorubicin and platinum are currently one combination being utilized by cooperative groups. Thus the current study seeks to determine the value of platinum when added to cyclophosphamide, doxorubicin and VP 16.

As of April, 1980, 20 patients have been entered onto the cyclophosphamide, CCNU, doxorubicin, VP 16-213 + platinum study. There have been 1 complete and 3 partial responders. There are too few patients so far to analyze by treatment, tumor subtype or prognostic factors, although response and survival will be analyzed by these criteria.

Proposed Course: Continue investigation.

Publications:

1. Hankins, J.R., Satterfield, J.R., Aisner, J., Wiernik, P.H., McLaughlin, J.S.: Pericardial window for malignant pericardial effusion. Ann. Thorac. Surg. (In press).
2. Aisner, J.: Lung Cancer. In Wiernik, P.H. (Ed.): The Science and Practice of Clinical Medicine. New York, Grune & Stratton, Inc., 1980 (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 CM 06976-03 COB	
PERIOD COVERED October 1, 1979 through September 30, 1980					
TITLE OF PROJECT (80 characters or less) Augmentation of Alkylating Agents with DMSO					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI: Joseph Aisner, M.D.		Head, Section of Medical Oncology		COB NCI	
Others: Peter H. Wiernik, M.D.		Chief, Clinical Oncology Branch		COB NCI	
Stanley Ostrow, M.D.		Senior Investigator		COB NCI	
Merrill Egorin, M.D.		Scientific Expert		LCB NCI	
Nicholas Bachur, M.D., Ph.D.		Chief, Laboratory of Clinical Biochemistry		LCB NCI	
COOPERATING UNITS (if any) None					
LAB/BRANCH Clinical Oncology Branch					
SECTION Section of Medical Oncology					
INSTITUTE AND LOCATION NIH, NCI Baltimore, MD 21201					
TOTAL MANYEARS: 0.5		PROFESSIONAL: 0.5		OTHER: 0	
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) A number of advanced cancers such as renal cell carcinoma and squamous cell carcinoma of the lung have proven particularly refractory to chemotherapy. It is therefore warranted to test new agents or new experimental modalities in these tumors. Animal studies suggest that the chemotherapeutic activity of certain alkylating agents can be augmented by the use of dimethylsulfoxide (DMSO). Patients with metastatic squamous cell carcinoma were therefore tested with DMSO and cyclophosphamide. Ten patients with squamous cell lung cancer have been started on DMSO plus cyclophosphamide. There have been no objective anti-tumor responses seen in these patients, but it is too early to determine overall activity of this combination. Pharmacokinetics have been initiated to determine if the DMSO changes the disposition of the cyclophosphamide. So far no differences have been noted where each patient serves as his/her own control.					

There have been many advances in the drug treatments of a variety of human malignancies. Several tumors such as metastatic renal cell carcinoma and advanced squamous cell carcinoma of the lung have proven relatively refractory to conventional drug therapy. Therefore, it is warranted to test new agents or newer experimental modalities for the treatment of these diseases. It has recently been demonstrated that the anti-tumor activity of several antineoplastic drugs, especially the alkylating agents, can be enhanced by the administration of oral dimethylsulfoxide. Patients with advanced squamous cell carcinoma of the lung were given dimethylsulfoxide in water orally and then treated with intravenous cyclophosphamide to test whether this treatment might increase the permeability of the drug and augment the response that might be seen from cyclophosphamide. Pharmacokinetics were performed to follow drug disposition.

Ten patients with advanced squamous cell carcinoma of the lung have been started on escalating doses of dimethylsulfoxide in water, starting at 2% and going to 5%, followed by intravenous cyclophosphamide to determine if the low order of activity of this drug might be enhanced. In order to determine drug levels and levels of drug-inclosed body fluids, samples of blood, urine, and cerebrospinal fluid are assayed for parent compound and cyclophosphamide metabolites. Patients receive cyclophosphamide alone once as a control for pharmacokinetics. It is too early to determine if there is any augmentation of any effect on response rate or survival. Although so far no responses have been seen. In addition there are no apparent differences in cyclophosphamide disposition as yet.

Proposed Course: Continue investigation.

NIHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09113 02 COB														
PERIOD COVERED October 1, 1979 through September 30, 1980																
TITLE OF PROJECT (80 characters or less) Treatment of Advanced Gynecological Malignancies																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 40%;">Dean E. Brenner, M.D.</td> <td style="width: 40%;">Clinical Associate</td> <td style="width: 10%; text-align: right;">COB NCI</td> </tr> <tr> <td rowspan="3">Others:</td> <td>Joseph Aisner, M.D.</td> <td>Chief, Section Medical Oncology</td> <td style="text-align: right;">COB NCI</td> </tr> <tr> <td>Philip Konits, M.D.</td> <td>Clinical Associate</td> <td style="text-align: right;">COB NCI</td> </tr> <tr> <td>Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td style="text-align: right;">COB NCI</td> </tr> </table>			PI:	Dean E. Brenner, M.D.	Clinical Associate	COB NCI	Others:	Joseph Aisner, M.D.	Chief, Section Medical Oncology	COB NCI	Philip Konits, M.D.	Clinical Associate	COB NCI	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
PI:	Dean E. Brenner, M.D.	Clinical Associate	COB NCI													
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	Philip Konits, M.D.	Clinical Associate	COB NCI													
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI													
COOPERATING UNITS (if any) Division of Gynecological Oncology, Dept. OB/GYN, University of Maryland Hospital Hospital de Clinicas "Dr. Manuel Quintela", Montevideo, Uruguay																
LAB/BRANCH Clinical Oncology Branch																
SECTION Section of Medical Oncology																
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201																
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">0.5</td> <td style="text-align: center;">0.5</td> <td style="text-align: center;">0</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	0.5	0.5	0								
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:														
0.5	0.5	0														
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																
SUMMARY OF WORK (200 words or less - underline keywords) Although alkylating agents are active, combination of chemotherapy is superior to single agent therapy in patients with advanced <u>ovarian cancer</u>. <u>Doxorubicin</u> and <u>cis-platinum</u> is an active combination. <u>VP16-213</u> has activity in male germinal tumors. The combination of <u>cis-platinum</u>, <u>doxorubicin</u>, <u>cyclophosphamide</u> and <u>VP16-213</u> creates the possibility of multiple synergistic pairs. This 4 drug combination is therefore studied for the treatment of advanced epithelial tumors of the ovary. Two patients have been entered into study. There is suggestion of antitumor activity with partial responses in both patients who had advanced high grade tumors. It is too early to evaluate for response or toxicity. Salvage chemotherapy in patients with <u>ovarian carcinoma</u>, <u>endometrial carcinoma</u>, or <u>cervical carcinoma</u> rarely exceeds 10% to 20% response rates. In the current study 22 patients with advanced gynecologic malignancies, 8 ovary, 9 cervix, 2 endometrial, and 3 adenocarcinomas, have been treated with 120 mg/m² of <u>MAMS</u>. Two partial responses, one in endometrial carcinoma and one in cervical carcinoma have been observed in 20 evaluable patients. It is still too early to evaluate level of activity since at least 15 patients per tumor type are needed to exclude a 20% possibility of activity.																

Recent studies have demonstrated the superiority of multiagent chemotherapy to single agent chemotherapy in patients with advanced ovarian carcinoma. Cis-platinum has established activity as a single agent in this disease. Vp16-213 has activity in male germinal tumors and has shown increased activity with doxorubicin in small cell lung tumors and breast carcinoma. Doxorubicin and cis-platinum have shown enhanced activity in patients with ovarian carcinoma. The combination of doxorubicin and cyclophosphamide is widely used in multiple tumors with demonstrated synergism. The combination of cis-platinum, doxorubicin cyclophosphamide, and vp16-213 creates the possibility of multiple synergistic pairs.

Each of these drugs has different toxicities, thus nonoverlapping toxicities when given in combination. This 4 drug combination is undertaken as a pilot study to determine response rates and toxicity in patients with advanced epithelial ovarian carcinoma. Two patients were entered into study. There is suggestion of antitumor activity with partial response in the two patients. Both patients have high grade, advanced stage tumors. There has been significant myeloid toxicity from this combination in both patients studied. One patient developed azotemia when cis-platinum was given in a low-renal perfusion state. However, with only two patients entered, it is impossible to make any conclusions about this combination.

PROPOSED COURSE: Continue investigation

Tumors of the female genital tract constitute 20% of the overall cancer incidence in the females. Salvage chemotherapy remains poor. Patients with carcinoma of the ovary, carcinoma of the cervix, or carcinoma of the endometrium, following initial treatment, rarely respond to second line chemotherapy. Therefore, the introduction of new cytotoxic agents and their testing in this group of patients is important to add to current chemotherapeutic armamentarium.

MAMSA is one of a series of acridines synthesized in an effort to design agents which would bind to DNA and would have antitumor activity. Current phase II studies with gynecologic tumors are in progress using 120 mg/m² Q 3 weeks.

PROJECT A: Carcinoma of the Ovary.

Eight patients with carcinoma of the ovary have been entered on this protocol. All were patients with late-stage disease heavily treated with chemotherapy. No responses were observed. There was no response to MAMSA and there was severe myelotoxicity in 4 of the patients studied and mild gastrointestinal toxicity in 2 patients. More patients are needed to complete this study.

PROPOSED COURSE: Continue investigation

PROJECT DESCRIPTION

Z01 CM 09113 02 COB

PROJECT B: Carcinoma of the Cervix.

Nine patients with advanced carcinoma of the cervix were entered on this protocol. One patient responded. Six were treated with prior radiation and/or chemotherapy. There was myelotoxicity in approximately 25% of studied patients and mild gastrointestinal toxicity. No other significant toxicity was observed. More patients need to be entered in order to exclude the 20% possibility of response.

PROPOSED COURSE: Continue investigation

PROJECT C: Carcinoma of the Endometrium.

Two patients with advanced carcinoma of the endometrium have been entered on this study. One patient responded. No toxicity has been observed. More patients need to be entered on protocol in order to satisfactorily assess the activity of this drug.

PROPOSED COURSE: Continue investigation

PROJECT D: Adenosquamous Carcinoma.

Three patients with adenosquamous carcinoma of the endocervix have been entered on study. No responses have been observed. Minimal toxicity, other than moderate myelotoxicity has been observed. Further patient accumulation in this tumor is needed to assess drug activity.

PROPOSED COURSE: Continue investigation

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 CM 09117 02 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Phase II Studies With New Agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	David A. Van Echo, M.D.	Senior Investigator COB NCI
Others:	Peter H. Wiernik, M.D. Joseph Aisner, M.D. Joachim Fuks, M.D. Phillip Konits, M.D. Susan Markus, R.N. Nicholas Bachur, M.D. Merrill Egorin, M.D.	Chief, Clinical Oncology Branch COB NCI Head, Section Medical Oncology COB NCI Clinical Associate COB NCI Clinical Associate COB NCI Chemotherapy Nurse COB NCI Chief, Lab. Clin. Biochemistry COB NCI Expert, Lab. Clin. Biochemistry COB NCI
COOPERATING UNITS (if any) Hospital de Clinicas "Dr. Manuel Quintela", Montevideo, Uruguay		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Medical Oncology		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES)		
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Phase II studies are therapeutic investigations of new agents that have finished Phase I testing (I.E. clinical toxicology and pharmacology studies). Completed negative Phase II trials have been done with <u>AMSA</u> in oat cell carcinoma of the lung. Trials are currently underway with intravenous <u>6-thioguanine</u> in colorectal carcinoma; <u>AMSA</u> in malignant melanoma and <u>Methy-G</u> in renal and GU tumors and esophageal carcinoma; and <u>Neocarzinostatin</u> plus <u>VP16-213</u> in hepatocellular carcinoma.		

Phase II studies are therapeutic trials of new anticancer agents that have finished clinical pharmacokinetic and toxicity testing (Phase I trial). A minimum of 14 patients with refractory metastatic malignant disease are entered in each disease category to exclude a possible 15-20% response rate with 95% confidence limits. Patients are expected to have measurable disease and an expected survival of two months. If responses are seen additional patients are entered to adequately define a response rate.

PROJECT A Colorectal Carcinoma

Intravenous 6-Thioguanine in colorectal carcinoma. Six patients have been entered on study at a dose of 800-1000 mg/m² IV bolus every three weeks. The study is too early for response data. Toxicities are emesis and leukopenia. A method has been devised using a high pressure liquid chromatography technique to measure parent compound and pharmacokinetics.

Conclusion: Study too early to evaluate.

PROJECT B Renal Cell Carcinoma

AMSA in Renal Cell Carcinoma: Sixteen patients were treated with AMSA at 120-150 mg/m² as a one hour infusion every three weeks. No antitumor responses were seen. Toxicities were leukopenia and phlebitis.

Conclusions: Study closed. AMSA is ineffective in renal cell carcinoma.

PROJECT C Oat Cell Carcinoma of the Lung

AMSA in Oat Cell Carcinoma (OCC): Eighteen patients with relapsed OCC of the lung received AMSA at 120-140 mg/m² as a one hour infusion every three weeks. No antitumor responses were seen. One patient died from sepsis during a period of AMSA-induced granulocytopenia.

Conclusions: Study closed. AMSA is ineffective in relapsed oat cell carcinoma of the lung.

PROJECT D Malignant Melanoma

AMSA in Malignant Melanoma: Six patients have been entered on study and treated at a starting dose of 150 mg/m² every three weeks. The study is too early to evaluate for response. No significant toxicities have been seen suggesting that malignant melanoma patients have more bone marrow tolerance for treatment with myelotoxic agents.

Conclusions: Too early to evaluate

PROJECT E GU Malignancies

Methyl-G in Renal and GU Malignancies: Seven patients with renal cell carcinoma have been entered on study at 5-600 mg/m² of week. No responses have been seen. One patient with transitional cell carcinoma of the renal pelvis has achieved a partial response (PR). Toxicities have been diarrhea and neuromuscular.

Conclusions: Too early to evaluate for response. Continue study.

PROJECT F Esophageal Carcinoma

Methyl-G in Esophageal Carcinoma: Study recently activated and no patients have been entered yet. To be done in collaboration with the Latin American-USA Exchange.

PROJECT G Hepatoma

NCS + VP 1b-213 in Hepatoma: Seven patients have been entered on-study. Survival on this study is directly related to pretreatment performance status. Although no significant tumor regressions have been seen, stabilization has occurred in several patients for as long as 12 months.

Conclusions: Too early to evaluate for response. Study open.

Proposed Course: Continue investigation.

Publications:

1. Van Echo, D.A., Diggs, C.H., Scoltock, M., Wiernik, P.H.: Phase II evaluation of PALA (N-phosphono-acetyl-L-aspartase, NSC 2224131) in metastatic adenocarcinoma of the colon or rectum. Cancer Treatment Reports (in press), 1980.
2. Van Echo, D.A., Markus, S., Aisner, J., Wiernik, P.H.: A phase II trial of AMSA (NSC 249992) in patients with metastatic renal cell carcinoma. Cancer Treatment Reports, (in press), 1980.
3. Fuks, J.Z., Van Echo, D.A., Aisner, J., Von Hoff, D., Wiernik, P.H.: A phase II of 4'-(9-acridinylamino)-methanesulfon-m-aniside (AMSA) in patients with refractory small cell carcinoma of the lung (SCCL). Cancer Treatment Reports (in press), 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09123-01 COB												
PERIOD COVERED October 1, 1979 through September 30, 1980														
TITLE OF PROJECT (80 characters or less) Evaluation of Biomarkers in Patients with Advanced Malignant Disease														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Joseph Aisner, M.D.</td> <td style="width: 33%;">Head, Section of Medical Oncology</td> <td style="width: 33%;">COB NCI</td> </tr> <tr> <td>Others: Margaret A. Whitacre, R.N.</td> <td>Hyperthermia Nurse</td> <td>COB NCI</td> </tr> <tr> <td>David A. Van Echo, M.D.</td> <td>Senior Investigator</td> <td>COB NCI</td> </tr> <tr> <td>Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td>COB NCI</td> </tr> </table>			PI: Joseph Aisner, M.D.	Head, Section of Medical Oncology	COB NCI	Others: Margaret A. Whitacre, R.N.	Hyperthermia Nurse	COB NCI	David A. Van Echo, M.D.	Senior Investigator	COB NCI	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
PI: Joseph Aisner, M.D.	Head, Section of Medical Oncology	COB NCI												
Others: Margaret A. Whitacre, R.N.	Hyperthermia Nurse	COB NCI												
David A. Van Echo, M.D.	Senior Investigator	COB NCI												
Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI												
COOPERATING UNITS (if any) Raymond W. Ruddon, Jr., M.D., Ph.D., Head, Biological Markers Program, Frederick Cancer Research Center														
LAB/BRANCH Clinical Oncology Branch														
SECTION Section of Medical Oncology														
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201														
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:												
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CHECK APPROPRIATE BOX(ES)														
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER														
☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)														
Biomarkers when available allow for certain malignancies to be either diagnosed early, recurrences to be diagnosed early, or allow for sequential analysis for the evaluation of treatment. These parameters are usually more sensitive indicators of disease than conventional diagnostic techniques. In an attempt to identify biomarkers from patients with advanced malignancies, blood samples were drawn at the time of admission and sequentially during treatment. Thirty-five patients have had multiple blood samples drawn for CEA, calcitonin, vasopressin, ACTH, and C3dp-C. Clinical correlation with biomarker data will be made to determine if there might be any relationship.														

Diagnostic techniques and technology allow for the evaluation of tumor masses only when they have accumulated to a sufficiently large degree to be either palpable or demonstrable radiographically. Such accumulations of tumor cells require a large number of cells and generally signify advanced disease. Biomarkers when available allow for the detection of tumor cells in much smaller deposits. Therefore, if biomarkers were available, one might diagnose tumors earlier, one might follow the results of treatment sequentially to determine when the patient's tumor burden has become sufficiently low, or one might use biomarkers to determine when a patient relapses.

In order to identify biomarkers, patients with advanced cancer were followed sequentially with serial blood samples and the results of the biomarkers assayed, then correlated with clinical status, treatment courses, time of relapse and other parameters.

As of April 1980, 35 patients have been sequentially tested for biomarkers. There were 24 patients with small cell lung cancer, 8 patients with adenocarcinoma of the lung and 2 patients with squamous lung cancer, and 1 patient with mixed lung primaries. Blood samples were assayed for CEA, calcitonin, vasopressin, ACTH and a complement fragment C3dp-C. There has been a wide variation in the results from tests. For example, CEA's have varied from 0 to 450 units. These ranges of biomarkers will be correlated with patients' clinical status, treatment, times and other clinical parameters to determine if there might be a relationship between levels of biomarkers and clinical course.

Proposed Course: Continue investigation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09135 01 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Adjuvant Combination Therapy of Resectable Adenocarcinoma of the Colon with Whole-Liver Irradiation and 5-Flourouracil		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Richard S. Kaplan, M.D. Senior Investigator COB NCI		
COOPERATING UNITS (if any) Division of Radiation Therapy, University of Maryland Hospital, Baltimore, MD All member institutions of Gastrointestinal Tumor Study Group		
LAB/BRANCH Clinical Oncology Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER		
SUMMARY OF WORK (200 words or less - underline keywords) A prospective randomized controlled study of liver-directed and systemic <u>adjuvant therapy</u> in patients with high risk <u>colon</u> (excluding rectal) <u>cancers</u> is being carried out by the Gastrointestinal Tumor Study Group. Patients are randomized to no adjuvant therapy or to 2100 R of <u>liver irradiation</u> combined with 3 days of IV 5-FU. Forty patients have been entered since the study was begun in August, 1979. No conclusions regarding efficacy can be made at this early date. Myelosuppression has been more striking than expected and, though no deaths have occurred, the 5-FU dose has been modified.		

Hepatic metastases have been well established as the leading cause of failure after surgical treatment of cancer of the large bowel proximal to the rectum. Various types of single agent adjuvant systemic chemotherapy have been tested with minimal or no success. Trials of combination therapy are underway or in follow-up. The efficacy of these regimens is as yet unknown. However, the ongoing trial of liver-directed adjuvant chemotherapy (portal vein infusion of 5-FU) by Taylor and associates in England has yielded encouraging early results.

This study is being undertaken by members of the Gastrointestinal Tumor Study Group to investigate another type of liver-directed (and systemic) adjuvant therapy. Whole-liver irradiation to 2100K plus simultaneous and subsequent IV 5-FU chemotherapy is being compared in a randomized fashion to no adjuvant therapy in patients with Dukes B₂ or C colon tumors following potentially curative surgery.

Thus far 40 patients have been entered since August 1979. Only 10 patients on the experimental arm have completed therapy thus far. No analysis of therapeutic results can be made at this early date.

However, some assessment of toxicity can be made. So far no evidence of serious hepatotoxicity has been seen, but myelosuppression from 5-FU has been apparently potentiated by the radiation. Eight patients have had WBC 1500 and/or platelet counts 70,000. Two patients have required hospitalization, one of whom was septic. She recovered and no patient has died. The starting dose of 5-FU has subsequently been reduced to minimize this problem.

PROJECTED COURSE: This study will continue to accrue patients to a total of 200 (2-3 years).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09137 01 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Evaluation of 9-THC as an Antiemetic in Patients with Malignancies Undergoing Chemotherapy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Joachim Z. Fuks, M.D. Others: Peter H. Wiernik, M.D. Joseph Aisner, M.D. Nathan Schnaper, M.D. Nicholas Bachur, M.D., Ph.D. Merrill J. Egorin, M.D. Dean Brenner, M.D. Charles Riggs, M.D.	Clinical Associate Chief, Clinical Oncology Branch Head, Section Medical Oncology Chief, Section Psychiatry Chief, Laboratory Clinical Biochemistry Scientific Expert Clinical Associate Clinical Associate	COB NCI COB NCI COB NCI COB NCI LCB NCI LCB NCI COB NCI LCB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Medical Oncology		
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Nausea and vomiting are frequent and distressing side effects of cancer chemotherapy and the severity of these symptoms contributes to the decreased ability of patients to undergo long-term chemotherapy. The phenothiazines have marginal value for nausea and vomiting and more effective antiemetic therapy is needed. THC has recently been identified to contain significant antiemetic potential. This randomized, double-blind, placebo controlled study compares the antiemetic effectiveness of ⁹ THC with a standard phenothiazine-antiemetic, prochlorperazine. Furthermore, since THC can induce tolerance to THC this study also examines whether ⁹ THC alters drug handling of the chemotherapeutic agents.		

Nausea and vomiting, an almost universal side effect of chemotherapy, remains a major problem to patients, not infrequently causing them to withdraw from treatment programs. Currently employed drugs for achieving anti-emesis, including phenothiazines, often fail to ameliorate chemotherapy-related nausea and vomiting. ⁹-THC, the active ingredient in marihuana has been identified as possessing antiemetic effect. THC can be administered orally or by inhalation. Several reports have suggested that THC:

1. is a more effective antiemetic agent than Compazine
2. can induce tolerance, thus subsequent THC dose may need to be increased
3. may stimulate induction of enzymes
4. may have drug interactions with chemotherapeutic agents

Patients, ages 21 to 65 who receive chemotherapy with cis-platinum; adriamycin and cyclophosphamide alone or in drug combinations are allowed to participate. Patients are randomized in double blind fashion to receive ⁹-THC, Compazine or placebo. Patients give information on nausea, vomiting, sedation, incoordination, "high". During this study blood samples are drawn periodically to study plasma level of ⁹-THC and pharmacokinetics of adriamycin, cyclophosphamide and/or cis-platinum.

As of April 1980 five patients have been evaluated. Some anti-emetic effect has been noted but because of a small patient group the code has not been broken yet and the assessment of exact anti-emetic efficacy of THC as well its plasma levels and interactions with chemotherapeutic agents is not yet evaluable.

Proposed Course: Continue investigation

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09139 01 COB												
PERIOD COVERED October 1, 1979 through September 30, 1980														
TITLE OF PROJECT (80 characters or less) The Use of Computed Tomographic Scanning in Oncology														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">Pl:</td> <td style="width: 40%;">Dean E. Brenner, M.D.</td> <td style="width: 40%;">Clinical Associate</td> <td style="width: 10%;">COB NCI</td> </tr> <tr> <td>Others:</td> <td>Joseph Aisner, M.D.</td> <td>Chief, Section Medical Oncology</td> <td>COB NCI</td> </tr> <tr> <td></td> <td>Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td>COB NCI</td> </tr> </table>			Pl:	Dean E. Brenner, M.D.	Clinical Associate	COB NCI	Others:	Joseph Aisner, M.D.	Chief, Section Medical Oncology	COB NCI		Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI
Pl:	Dean E. Brenner, M.D.	Clinical Associate	COB NCI											
Others:	Joseph Aisner, M.D.	Chief, Section Medical Oncology	COB NCI											
	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI											
COOPERATING UNITS (if any) Department of Radiology, University of Maryland Hospital, Baltimore, MD 21201														
LAB/BRANCH Clinical Oncology Branch														
SECTION Section of Medical Oncology														
INSTITUTE AND LOCATION NCI, NIH Baltimore, MD 21201														
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0												
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The advent of the <u>computed tomographic scan</u> has opened the possibility of <u>locating</u> , <u>biopsing</u> , and sequentially <u>analyzing</u> masses in previously inaccessible areas. To assess this technique, we have devised projects to prospectively study the capabilities and drawbacks of this technology. Fourteen patients with advanced <u>carcinoma of the cervix</u> have been evaluated preoperatively with good correlation for the presence of retroperitoneal adenopathy. Biopsies under CT guidance have provided adequate tissue in 75% of 24 patients studied. <u>Ovarian carcinomas</u> are being studied prospectively with 92% predictive accuracy. Other studies include serial assessments of other tumor, particularly lung tumors and tumor volume studies. Further patient entries will provide more meaningful data in all projects described.														

PROJECT A: Biopsy under CT Scan guidance.

Twenty-five patients have had biopsy or aspiration procedures under CT scan guidance. Six have had large gauge needle biopsy of liver nodules or abdominal masses without morbidity with adequate tissue and pathology obtained in five. Three of four aspirations of the head of the pancreas has obtained adequate information. Eleven abscess aspirations have yielded adequate data in 8 patients. Aspirations of the lung in two patients and of the kidney in two patients have yielded adequate material for diagnosis. Pathologic diagnosis was obtained in 75% of patients biopsied or aspirated. Another 10% have had useful data collected, although a pathologic diagnosis was not established. Further patients entries are needed.

PROPOSED COURSE: Continue investigation

PROJECT B: Pre-operative assessment staging and node status in patients with advanced carcinoma of the cervix.

All patients with advanced carcinoma of the cervix (Stage IIA-IVA) require retroperitoneal node sampling in order to determine the location of radiotherapy portals for the possibility of curative radiation or surgery. Fourteen patients with advanced carcinoma of the cervix had abdominal and pelvic CT scans performed prior to surgery. Early results reveal: 2 true positive, 1 false positive, 8 true negative and no false negative. CT Scanning missed microscopic invasion into adjacent visceral pelvic structures (e.g. bladder and rectum). Additional patients are needed to confirm these early results.

PROPOSED COURSE: Continue investigation

PROJECT C: response to therapy as documented by CT scan in patients with solid tumors.

Computed tomography has rendered accessible, areas of the body that previously could only be studied by invasive techniques, if at all. Lesions of lungs, pleura, mediastinum which are not visible on routine chest films may be detected on CT scan. Patients on protocols treating bladder, testicular, lung, and soft tissue sarcomas are being scanned prior to therapy, then sequentially during treatment. Early data suggest that CT scans may determine response. More follow-up data is necessary to document the accuracy of this technique.

PROPOSED COURSE: Continue investigation

PROJECT D: response to chemotherapy as documented by CT scan in patients with ovarian carcinoma.

PROJECT DESCRIPTION

201 09139 01 COB

because of its deep abdominal location, results of therapy in patients with carcinoma of the ovary have been difficult to determine. Second or third look laparotomies are commonly necessary to determine response to chemotherapy. Thirteen patients have had abdominal and pelvic CT scans performed prior to chemotherapy and were followed for response to therapy. There was a 92% predictive accuracy in determining response to therapy. In many cases progressions were predicted prior to clinic signs or other evidence. Further patient accrual is still needed.

PROPOSED COURSE: Continue investigation

PROJECT E: Estimation of tumor volumes by CT scanner.

Measurement of tumor response to therapy by standard roentgenographic or nuclide scan techniques is fraught with measurement errors. The CT scan allows the determination of sequential crosssectional areas, allowing computation of the volume of a given organ or mass. Current studies determine volume of organs such as spleens in patients who are about to have the organ removed electively. Additional volume estimates of human organs from cadavers are in progress. Studies are underway to determine sources of error and refine CT techniques of volume estimation.

PROPOSED COURSE: Continue investigation

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 CM 06217-11 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Prevention of Infections in Patients with Cancer		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	S.C. Schimpff Head, Section of Infection Research	COB NCI
Other:	K.A. Newman Infection Control Nurse	COB NCI
	J.C. Wade Clinical Associate	COB NCI
	P.H. Wiernik Chief, Clinical Oncology Branch	COB NCI
	C.L. Fortner Head, Clinical Research Pharmacy	COB NCI
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Infection Research		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 8	PROFESSIONAL: 6	OTHER: 2
CHECK APPROPRIATE BOX(ES)		
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection. At the Baltimore Cancer Research Program an intensive <u>infection surveillance program</u> assesses the ongoing infectious disease status of the patients and the Program as a whole. From this data base have grown appropriate basic <u>infection control policies</u> and specific research approaches including evaluation of <u>laminar air flow rooms</u>, simpler means of air filtration and reverse isolation, <u>oral nonabsorbable antibiotics</u> and techniques to prevent axillary and oral infections. <u>Trimethoprim/sulfamethoxazole</u>, an absorbable antibiotic, is being evaluated as a means of suppressing the aerobic gram-negative flora while preserving <u>colonization resistance</u>. These programs are enforced by the <u>infection control nurse</u>.		

Infection remains as a major cause of both morbidity and mortality for patients with various types of cancers. The risk of infection increases as new techniques are introduced into medical practice which potentially damage host defense mechanisms and as greater numbers of patients are treated with more intensive cytotoxic and immunosuppressive therapy. It is therefore essential in the modern practice of oncology that definitive approaches be designed and evaluated to reduce the opportunities for infection.

Specific Projects:

- A. Surveillance - The cornerstone of an infection control program is an accurate understanding of the infections presently occurring including the sites and etiologic agents, apparent predisposing factors, routes of acquisition of hospital organisms, and changing patterns of morbidity and mortality. At the Baltimore Cancer Research Program, this is accomplished by the daily rounds of an infection control nurse, twice weekly infectious disease rounds, daily evaluation of all infected or potentially infected patients, and an extensive program of both patient and environmental microbial culturing. The data derived is summarized regularly and utilized to adjust, on a continuing basis, the programs in infection control.
- B. Basic Infection Control Procedures - The Infection Control Policy Manual has been continually updated with enforcement pursued by the infection control nurse. The following procedures are standard: 1) urinary, intravenous, and hyperalimentation catheters are avoided except under extenuating circumstances, 2) all intravenous solutions are prepared in laminar air flow hoods, all bottles and tubing are replaced daily, and butterfly needles are changed every other day, 3) personal hygiene is emphasized including handwashing and the use of special germicidal hand lotions, 4) all in patients are seen regularly by the infection control nurse for discussion of preventive techniques, 5) results of patient and environmental surveillance cultures are utilized to direct specific control techniques. These techniques have substantially reduced nosocomial infection.
- C. Oral Nonabsorbable Antibiotics - Controlled trials at the BCRP and elsewhere have indicated the usefulness of oral nonabsorbable antibiotics in reducing the incidence of severe infections arising along the alimentary tract and also in the lungs. Although not due solely to the oral antibiotics, the incidence of bacteremia has fallen from 13.3 per 1000 days to 3.2 per 1000 days over the past 5 years. However, the poor taste and frequent nausea and diarrhea leads to difficulties with compliance and hence efficacy. A series of comparative trials was therefore initiated comparing taste, tolerance, compliance, microbial suppression and infection prevention. Previously, it was found that tobramycin could be substituted for gentamicin and that vancomycin could be eliminated from the original GVN (gentamicin, vancomycin and nystatin) regimen.
- D. In an attempt to further 1) evaluate the preservation of "colonization resistance", 2) improve patient tolerance, and 3) reduce cost, relapsed acute leukemia patients were randomly allocated to receive either gentamicin plus nystatin (GN) or trimethoprim/sulfamethoxazole (TMP/SMZ) plus nystatin. Also, 40 patients with oat cell carcinoma of the lung who were to receive intensive myelosuppressive chemotherapy were randomly allocated to receive trimethoprim/sulfamethoxazole or a placebo. Extensive microbiologic studies of the patients with oat cell carcin-

oma indicated that TMP/SMZ does adequately suppress the aerobic gram-negative microflora of the alimentary canal without suppressing the anaerobic flora, presumably thereby preserving "colonization resistance". More patients will need to be studied to determine the full prophylactic effect but the trend is toward a reduction in gram-negative infections in the TMP/SMZ treated group. Also as of this date, 44 patients with acute leukemia have been evaluated. Those receiving TMP/SMZ have had rates of infection comparable to those receiving GN, indicating that TMP/SMZ+N is effective for infection prophylaxis in this patient population. Acquisition and infection with gentamicin-resistant gram-negative rods has been minimal. Patient tolerance and compliance are markedly improved. The cost of TMP/SMZ+N is much less than GN. These results indicate that this new approach to infection prevention may prove to be equally efficacious, yet less toxic, more tolerable and less expensive than total microbial suppression with oral nonabsorbable antibiotics.

- E. Based on the results above that TMP/SMZ+N is an effective form of infection prophylaxis and based on reports from elsewhere that nalidixic acid will, similarly to TMP/SMZ, suppress alimentary tract aerobes but not anaerobes, a new trial was initiated. Newly diagnosed, previously untreated patients with ANLL who are not infected at the time of admission are randomly allocated to TMP/SMZ+N or nalidixic acid plus nystatin (NA+N). Twenty-seven patients have been entered to date with initial results suggesting a possible advantage for the TMP/SMZ+N regimen with regard to infection prevention. However, allergic reactions do occur with sulfa compounds whereas nalidixic acid has no apparent significant side effects. Further evaluation with additional patients will be required.
- F. Laminar air flow room isolators have been recently installed at the BCRP.

A major problem is that newly admitted patients with ANLL who are infected at the time of admission have a reduced complete remission rate and consequently a reduced overall survival time. Remission rates are low (~30% compared to 80% for noninfected patients) because many of these patients die of infection before receiving an adequate trial of cytotoxic therapy. Death is usually due to the rapid occurrence of new infection, often from an acquired fungus or from a recently acquired antibiotic-resistant gram-negative bacillus.

Most of the careful evaluations of LAF isolation microbial suppression techniques have excluded from evaluation patients who presented with infection. This was done to ensure comparability between groups and to reduce confusion during evaluation. As a result, there is no clear understanding at the present time as to the degree of value of LAF isolation-microbial suppression for the patient admitted with pre-existing infection. It can be assumed however that this approach will substantially reduce the incidence of new infection and, as a result, it can be hypothesized that with reduced infection and reduced infectious death, the remission rate should increase and approach that of the patient admitted without infection. This protocol seeks to resolve this question by randomly allocating patients to the current standard approach at the BCRP (Control group) or to that same approach with the addition of complete reverse isolation within the laminar air flow room (LAF group). In both groups, the remission induction chemotherapy will be the same and supportive care will be equivalent with the only difference in the

management between the two groups being the utilization of total barrier reverse isolation nursing in a laminar air flow unit or not. This study is just now starting.

Additional evaluations in the LAF environment will include: comparison of remission induction potential for patients with ANLL over the age of 60 years. These patients have a poor response rate than younger patients because death often occurs because of supervening infection. It is possible that a substantial reduction in infection among these patients will result in an increased remission rate. A third group of patients are those in complete remission of ANLL who will be receiving intensive cytoreductive maintenance therapy. These patients require the maximum infection prevention available due to the high risk involved with this form of maintenance.

- G. Prophylactic Granulocyte Transfusions - In the setting of granulocytopenia, the prophylactic administration of granulocyte transfusions would appear logical. A prospective, randomized evaluation of granulocyte transfusions is discussed under the projects of the Section of Cell Component Therapy.
- H. The Effect of Chemotherapy and Oral Antibiotics on Intestinal Absorption - Since patients who are receiving cytotoxic therapy for acute leukemia or who are receiving prophylactic oral antibiotics for infection prevention frequently experience anorexia, nausea, vomiting, diarrhea and mucositis, it is possible that intestinal absorption may be impaired. Patients with acute leukemia about to receive cytotoxic therapy and prophylactic oral antibiotics are being studied prospectively to determine whether the chemotherapy, the oral antibiotics, both or neither have a substantial effect on intestinal absorption function. Fourteen ANLL patients have been studied before therapy, following cytotoxic therapy and following the use of oral prophylactic antibiotics. Evaluation has included serum vitamin A concentration, karotene, folate, and serum and urinary d-xylose. The results of the patients studied indicate that there is no major effect on intestinal absorption with the exception of a moderate reduction in d-xylose absorption following the institution of oral nonabsorbable antibiotics.

Publications:

1. Bender, J.F., Schimpff, S.C., Young, V.M., Fortner, C.L., Love, L.J., Brouillet, M.D. and Wiernik, P.H.: A comparative trial of tobramycin versus gentamicin in combination with vancomycin and nystatin for alimentary tract suppression in leukemic patients. Europ. J. Cancer 15:35-44, 1979.
2. Schimpff, S.C.: Infection prevention during granulocytopenia. In Remington, J.S. and Swartz, M.N. (Eds.): Current Clinical Topics in Infectious Diseases 1980. New York, McGraw-Hill Book Company, 1980, pp. 85-106.
3. Schimpff, S.C., Aisner, J. and Wiernik, P.H.: Infection in acute non-lymphocytic leukaemia: the alimentary canal as a major source of pathogens. In van der Waaij, D. and Verhoef, J. (Eds.): New Criteria for Antimicrobial Therapy: Maintenance of Digestive Tract Colonization Resistance. Amsterdam, Excerpta Medica, 1979, pp. 12-29.

4. Wade, J.C. and Schimpff, S.C.: Infections in patients with suppressed cellular immunity. In Klastersky, J. and Staquet, M. (Eds.): Supportive Care in Cancer Patients. II. New York, Raven Press, 1980 (in press)
5. Schimpff, S.C. and Young, V.M.: Epidemiology and prevention of infection in the immunocompromised host. In Young, L.S. and Rubin, R.H. (Eds.): Clinical Approach to Infection in the Immunocompromised Host. New York, Plenum Press 1980 (in press)
6. Schiffer, C.A., Aisner, J., Daly, P.A., Schimpff, S.C. and Wiernik, P.H.: Alloimmunization following prophylactic granulocyte transfusion. Blood 54:766-774, 1979.
7. Newman, K.A. and Schimpff, S.C.: Protective isolation: What policies and procedures are necessary? Asepsis 1:2-3, 1980.

INTERNATIONAL SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06264-11 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Evaluation of the Types and Causes of Infectious Diseases in Cancer Patients		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: S.C. Schimpff Other: K.A. Newman J.C. Wade P.H. Wiernik V.M. Young	Head, Section of Infection Research Infection Control Nurse Clinical Associate Chief, Clinical Oncology Branch Head, Research Microbiology Section	COB NCI COB NCI COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) D. Overholser, D. Peterson, Department of Oral Diagnosis, University of Maryland School of Dentistry; M. Applefeld, Division of Cardiology, University of Maryland School of Medicine		
LAB/BRANCH Clinical Oncology Branch SECTION Section of Infection Research		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies such as Hodgkin's disease and oat cell carcinoma of the lung. The aims of this project are to catalogue and identify the infections, <u>identify the predisposing factors</u> and evaluate new techniques for more rapid and/or accurate <u>diagnosis of infection</u> . This program has been in progress for over nine years and has allowed meaningful evaluation of the types of infection occurring in different types of tumor patients. Specific problems have been identified such as the nature of <u>hospital acquisition of potential pathogens</u> including bacteria and fungi. This epidemiologic data has allowed appropriate and logical development of infection prevention techniques and application of newer methods of infection diagnosis such that there has been a reduction of infection morbidity and mortality.		

Objectives:

Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aims of this project are to 1) catalogue and identify the infections manifest both clinically and postmortem, 2) identify the predisposing factors which have contributed to the specific type of infection, 3) evaluate new techniques for more rapid and/or accurate diagnosis of infection.

Beginning July 1, 1970, each patient with suspected infection has been seen by one of the investigators who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records are kept of history, examination, laboratory data, etc. Formal infectious disease rounds are held twice weekly to review each recognized infection, discuss management and evaluate how each infection might have been prevented. New evaluations completed, or in progress, this year are included below.

A. Staphylococcus aureus Infection

Endocarditis is said to commonly accompany S. aureus bacteremia. This organism also is a familiar cause of sepsis in patients with acute leukemia. To assess these associations as well as treatment and outcome, we reviewed the medical records of all patients at the BCRP with acute leukemia who had one or more positive blood cultures for S. aureus between January 1969 and September 1978. Of the 370 leukemics at risk, 32 patients had 37 episodes of S. aureus bacteremia. Twenty-four episodes (65%) occurred when granulocyte counts were $<100/\mu\text{l}$ and all but two episodes when granulocytes were $<1000/\mu\text{l}$. The median duration of therapy was 14 days intravenously plus 7 days orally, a total of 21 days. There were not clinical manifestations of endocarditis; autopsies were available in 21 of 28 patients who died and in no case was there evidence of endocarditis. No secondary foci of infection were identified at autopsy or during life. Because of the frequent occurrence of endocarditis in patients with S. aureus bacteremia, prolonged intravenous antibiotic therapy has often been recommended. Such therapy is associated with superinfection in patients with compromised host defenses. Since, in this series, these complications of S. aureus bacteremia in patients with acute leukemia appear to be rare, a shorter course of therapy is justified. This lack of endocarditis might be related to an impaired ability to form the sterile vegetation (composed of platelets, fibrin and leukocytes) required for S. aureus implantation. To test this hypothesis, rabbits have been rendered thrombocytopenic with adriamycin, and a polyethylene catheter has been placed across the aortic valve. The sterile vegetations found in untreated animals have not occurred in the thrombocytopenic rabbits. The next step will be to challenge both thrombocytopenic and nonthrombocytopenic catheterized rabbits with S. aureus by intravenous inoculum to determine if the lack of sterile vegetation will prevent development of endocarditis.

B. Evaluation of Periodontal Hygiene and Infection

Although periodontal and other infections are of considerable concern following cytotoxic therapy or radiation therapy, little is known of the development of such

lesions in the cancer patient. In a cooperative study with the Department of Oral Diagnosis at the University of Maryland School of Dentistry, each newly admitted patient with acute nonlymphocytic leukemia is evaluated by a dentist and a dental hygienist. Following this evaluation and completion of an objective written assessment of oral hygiene, each patient is randomly allocated to routine oral care (brushing and flossing) with or without a complete oral prophylaxis. Appropriate antitumor therapy is then begun and the patient is followed three days per week by the dental hygienist. Objective assessments are made on a weekly basis and correlated with other clinically derived data including presence or absence of infection. In the first 24 patients with acute leukemia evaluated, although none was initially aware of periodontal disease, all were found to have either moderate or severe chronic infection. Acute exacerbations requiring systemic antimicrobial therapy have occurred in seven of the control patients but in none of the patients randomized to intensive prophylaxis. Intensive dental prophylaxis substantially reduces the frequency of acute exacerbations of periodontal infection during periods of granulocytopenia.

- C. Hepatitis - Forty-two consecutive patients with ANLL who achieved a complete remission following induction therapy with daunorubicin and cytosine arabinoside were evaluated for the development of hepatitis. Hepatitis was defined as a transaminase elevation of 2.5 x normal on two consecutive determinations five days apart but occurring within 160 days of the first blood product transfusion. Hepatitis secondary to drugs or primary disease were excluded and serologic determinations for differentiation or viral hepatitis A or B, cytomegalovirus, herpes or Toxoplasma were performed. As part of leukemia therapy, patients were randomly allocated to receive or not receive levamisole as immunotherapy. Of the 42 patients with a complete remission, 31 developed nonA/nonB hepatitis, an additional two patients developed Hb_sAg positive hepatitis and the remaining nine patients had no hepatitis. There was no difference in the number of transfused blood product units given to the groups with or without hepatitis (72 versus 73 units per patient). The median survival for patients who develop nonA/nonB hepatitis was significantly longer (549 days) than was the survival of those patients who did not develop hepatitis (322 days). Patients who received levamisole and who developed nonA/nonB hepatitis had the longest median survival (767 days). Three patients presently remain in complete remission (1053+, 1190+, 1558+ days); each of these three had nonA/nonB hepatitis. Median peak SGOT levels correlated with the complete remission duration and total survival. Thus, hepatitis of the nonA/nonB type was found to be associated with an increase in the complete remission duration and the total survival of patients with acute nonlymphocytic leukemia.
- D. Staphylococcus epidermidis - At the BCRP, Staphylococcus epidermidis has been found to be an increasingly significant pathogen among granulocytopenic patients with acute leukemia. S. epidermidis is usually thought of as a contaminant when it is present in blood cultures, but in the past three years there has been an increasing frequency of proven septicemia and other significant infections caused by S. epidermidis. No bacteremias were noted prior to 1977, but an incidence of 2.0/1000 hospital days for patients with acute leukemia was noted in 1977 followed by 5.8 in 1978 and 7.6 through mid-1979. Prior to 1977, all S. epidermidis infections were skin related; since that time 15% were skin, 14% were

sino-pulmonary, 35% alimentary tract and 35% bacteremias of unknown origin. In attempting to determine the cause for the increased frequency of this infection, bacteriophage typing indicated that a common environmental source could not be implicated. There was no association with the use of intravenous catheters or the use of steel needles. No association could be found with changes in antileukemia cytotoxic therapy or the use of systemic antimicrobials. All bacteremias occurred in the setting of a granulocyte count $<100/\mu\text{l}$ in patients who had some degree of mucosal damage along the alimentary canal. The colonization index (mean quantitation of *S. epidermidis* from surveillance cultures) was found to be substantially higher for patients given gentamicin-nystatin prophylaxis (2.3) compared to those given gentamicin-vancomycin-nystatin (0.7). With the reinstitution of vancomycin, the bacteremia rate returned during the last six months of 1979 to 2.0/1000 days. Although most isolates of *S. epidermidis* have been cephalosporin susceptible, blood cultures have tended to remain continuously positive despite the use of full dosage of cephalothin or cefazolin. The substitution of vancomycin has produced sterile blood cultures, but infection resolution is frequently slow until granulocyte recovery occurs.

- E. Venous Access - Access to the venous system is essential for the management of patients with acute leukemia, however, the effects of cytotoxic agents, antibiotics such as amphotericin and cephalosporins and other agents all have sclerosing effects on peripheral veins often resulting in extreme difficulty with blood drawing or further therapeutic administrations. Two systems for improved venous access have been evaluated at the Baltimore Cancer Research Program. Arteriovenous fistula were placed in 28 patients requiring an average of 78 minutes for surgery. Fistula survival was generally short with an average of 12 days. Twenty-one fistula clotted with loss of function (8 clotted with loss of function in less than 24 hours from placement), although five fistula functioned adequately until the patient's death. Minimal infectious problems developed with only two episodes of incision cellulitis postplacement. Of the 28 fistula created, only 8 were considered to be of benefit to the patient. In another approach to venous access, large bore indwelling catheters (Hickman catheters) were placed via the subclavian vein in 41 patients. These catheters exit via subcutaneous tunnel in the midchest region reducing the opportunity for local infection. Most patients were markedly thrombocytopenic and granulocytopenic at the time of placement, yet insertion was completed in an average of 39 minutes with minimal complications. The median catheter functional duration has been 81+ days. Only six Hickman catheters required removal; two for mechanical failures, two for proven infection and two for suspected infection. Catheter-related bacteremias have occurred in two patients of which one was cleared with systemic antibiotics. Two catheter exit site infections have also occurred but have cleared with systemic antibiotics without the necessity of catheter removal. The catheters remained patent without clotting and have been useful for blood drawing, chemotherapy and other therapy administration, hyperalimentation plus central venous pressure monitoring and platelet pheresis. The use of Hickman catheters has proved to be a practical, safe, effective means for improved venous access.

Publications:

1. Schimpff, S.C.: Infection in patients with cancer. In Lichtman, M.A. and Wiernik, P.H. (Eds.): The Science and Practice of Clinical Medicine. New York, Grune and Stratton, 1980 (in press)
2. Mikulski, S.M., Love, L.J., Bergquist, E.J., Hargadon, M.T., Applefeld, M.M. and Mergner, W.: Aspergillus vegetative endocarditis and complete heart block in a patient with acute leukemia. Chest 76:473-476, 1979.
3. Wade, J.C. and Schimpff, S.C.: Infections in patients with suppressed cellular immunity. In Klastersky, J. and Staquet, M. (Eds.): Supportive Care in Cancer Patients. I. New York, Raven Press, 1980 (in press)
4. Voith, M.A., Lichtenfeld, K.M., Schimpff, S.C. and Wiernik, P.H.: Systemic complications of MER immunotherapy of cancer: pulmonary granulomatosis and rash. Cancer 43:500-504, 1979.
5. Schimpff, S.C.: Pseudomonas bacteremia in granulocytopenic cancer patients. J. Inf. Dis. 1980 (in press)

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	201 CM 06266-11 COB
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PERIOD COVERED

October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Therapy of Suspected Bacterial Infection in Granulocytopenic Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.C. Schimpff	Head, Section of Infection Research	COB NCI
Other:	J.C. Wade	Clinical Associate	COB NCI
	V.M. Young	Head, Research Microbiology Section	COB NCI
	C.L. Fortner	Head, Clinical Research Pharmacy	COB NCI
	M.R. Moody	Senior Investigator	COB NCI
	P.H. Wiernik	Chief, Clinical Oncology Branch	COB NCI
	K.A. Newman	Infection Control Nurse	COB NCI

COOPERATING UNITS (if any)

H. Standiford, Division of Infectious Diseases, University of Maryland School of Medicine

LAB/BRANCH

Clinical Oncology Branch

SECTION

Section of Infection Research

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:

2.5

PROFESSIONAL:

2.0

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) DEFINED

☐ (a1) MINORS

☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline key words)

A series of consecutive antibiotic trials for suspected sepsis in granulocytopenic cancer patients began in 1969 and continues to the present. Initial evaluations proved the usefulness of carbenicillin and gentamicin and later trials, including a large multiinstitutional trial, indicated that carbenicillin (ticarcillin) plus gentamicin had the best efficacy-to-toxicity ratio of the three possible two-drug combinations of carbenicillin (ticarcillin), gentamicin and cephalothin. The following two-drug combinations were therefore evaluated: ticarcillin plus gentamicin, ticarcillin plus amikacin and ticarcillin plus netilmicin. In 183 consecutive patient-trials, the efficacy was high and the toxicity minimal. With the addition of early granulocyte transfusions for poor risk patients, a response rate of over 90% was achieved. A new penicillin derivative, mezlocillin, with exceedingly broad activity was utilized alone in 50 consecutive patients but proved less than satisfactory in the absence of concomitant aminoglycoside therapy. Therefore, ticarcillin plus amikacin became standard therapy to which piperacillin (a new broad spectrum penicillin) plus amikacin and moxalactam (a new broad spectrum cephalosporin) plus amikacin have been compared with excellent results.

Despite improvements in preventive techniques, infections remain as one of the most common serious complications of cancer therapy. The BCRP Section of Infection Research has continuing programs designed to improve the therapeutic approach to these infections.

A. In vitro evaluation of new antibacterial antibiotics.

1. Three new semisynthetic penicillins have become available for investigational use. Piperacillin, mezlocillin and azlocillin are substantially more effective in vitro than ticarcillin or carbenicillin against clinical isolates of Klebsiella pneumoniae, Streptococcus faecalis, Staphylococcus aureus, Serratia marcescens and Escherichia coli. In each of these instances, piperacillin was most active followed by mezlocillin followed by azlocillin. For Pseudomonas aeruginosa, piperacillin is most active followed by azlocillin followed by mezlocillin which is equivalent to ticarcillin, followed by carbenicillin. These evaluations prompted clinical trials of mezlocillin and piperacillin; azlocillin will be studied later in 1980.
2. Three new cephalosporin derivatives have been introduced: cefatoxime, moxalactam and cefaperazone. Unlike currently marketed cephalosporins, these three all have excellent in vitro activity against Pseudomonas aeruginosa in addition to most other gram-negative and gram-positive organisms. These evaluations have led to the institution of a new clinical trial comparing the standard ticarcillin plus amikacin to moxalactam plus amikacin for initial empiric therapy of granulocytopenic febrile patients with cancer.
3. A combination utilizing one drug from among the penicillins described above (i.e., piperacillin, mezlocillin or azlocillin) and one from among the cephalosporins (moxalactam, cefaperazone or cefatoxime) would have a very broad spectrum and, for most of the organisms that commonly cause infection during granulocytopenia, both drugs would be active. However, there is a possibility for antagonism with two β -lactam type antibiotics utilized together (e.g., piperacillin's activity against P. aeruginosa is antagonized by cefoxitin and cefamandole). The various combinations are therefore being tested in vitro for possible synergy or antagonism against over 500 recent isolates of gram-negative and gram-positive bacteria. To date, no antagonism has been detected.
4. In collaboration with Dr. Harold Standiford at the University of Maryland, sera from patients at the BCRP who received amikacin plus either ticarcillin, piperacillin or moxalactam are being tested for β -lactam concentration, aminoglycoside destruction, sera killing (cidal) activity against the patient's pathogen and sera killing activity against a battery of 60 stock gram-negative and gram-positive bacteria. To date, it appears that ticarcillin but not piperacillin or moxalactam will degrade the aminoglycosides, gentamicin and tobramycin but not amikacin, in vitro. Piperacillin plus amikacin has greater killing activity than ticarcillin plus amikacin against most gram-negative bacilli including P. aeruginosa, K. pneumoniae and E. coli whereas moxalactam plus amikacin has greater activity against S. aureus and K. pneumoniae than ticarcillin plus amikacin. Overall, the combination of piperacillin plus amikacin would be best in settings where P. aeruginosa was common whereas

moxalactam plus amikacin would be better in institutions where K. pneumoniae or S. aureus was common. Both of these regimens appear to be superior to ticarcillin plus amikacin.

B. Empiric Antibiotic Therapy for the Febrile Granulocytopenic Patient - The setting of new fever during granulocytopenia represents infection in ~60% of cases with bacteremia (especially gram-negative bacteremia) occurring in 20% thus necessitating the prompt empiric use of broad spectrum bactericidal antibiotics.

1. Ticarcillin, an analog of carbenicillin, has been used in over 500 patient-trials at the BCRP. It has been found to be equally efficacious to carbenicillin but, because it can be utilized at one half the dosage of carbenicillin, has less dose-related side effects. As a result, ticarcillin has supplanted carbenicillin, and ticarcillin in combination with amikacin has become the standard of therapy to which other approaches are current being compared.
2. Cephalothin plus gentamicin (or other aminoglycoside such as tobramycin or amikacin) is the most commonly used antimicrobial combination in the United States today but results of the BCRP prospective evaluations and two concurring randomized trials have indicated that these combinations have a high degree of nephrotoxic potential (~10%) particularly in the older patient with reduced renal function (~25%). Combinations of ticarcillin plus gentamicin, amikacin or netilmicin, compared prospectively in 162 febrile episodes, found these combinations to be only rarely nephrotoxic (< 5%) with all creatinine changes to be rapidly reversible.
3. In order to further evaluate the potential for cephalosporin-aminoglycoside nephrotoxicity and to determine whether or not the addition of a cephalosporin to the ticarcillin-aminoglycoside regimen would improve efficacy, a multiinstitutional trial was begun comparing ticarcillin plus amikacin to ticarcillin plus amikacin plus cefazolin. As of this time, 150 BCRP patient-trials have been entered to the group study of 900 patients. Nephrotoxicity has been minimal (<5%) in both groups. Either the use of cefazolin in replacement for cephalothin or the addition of ticarcillin appears to have led to this low order of renal damage. As a result, when a three-drug combination of cephalosporin, penicillin and aminoglycoside is warranted, the cefazolin, ticarcillin and amikacin combination would produce the best efficacy with the least side effects.
4. Ticarcillin and gentamicin sera levels were measured one and five hours after intravenous administration of antibiotics to 64 and 55 patients respectively. Patients receiving ticarcillin plus gentamicin had a significantly lower mean gentamicin level one and five hours after antibiotic administration than patients receiving cephalothin plus gentamicin. These unexpectedly low antibiotic serum levels in patients with serious infections treated with these antibiotic combinations are of potential clinical significance and indicate the need to determine serum antibiotic concentrations (especially of the aminoglycoside) in patients with normal creatinines while being treated with combinations such as ticarcillin plus gentamicin.

5. Mezlocillin is a new carbenicillin-like ureidopenicillin with a wide spectrum encompassing most of the gram-negative organisms which cause infection in these patients. Because of this broad spectrum, it was tested as a single agent in 50 consecutive patient-trials requiring empiric antibiotic therapy. Only 50% of the documented infections improved. The development of resistance to mezlocillin was rapid. Although toxicities were minimal, this agent is not effective as single drug therapy for these seriously ill granulocytopenic patients but would be expected to be effective when utilized in combination with an aminoglycoside.
6. Piperacillin is a new broad penicillin with a much broader spectrum than ticarcillin against gram-negative bacilli. It has the safety of penicillin plus their rapid bactericidal mode of action. Therefore, piperacillin plus amikacin was compared to ticarcillin plus amikacin in a randomized, prospective double blind trial including 128 febrile episodes in neutropenic patients. The response rates were equivalent despite the broader spectrum of piperacillin. Surprisingly, hypokalemia occurred as frequently among the piperacillin as the ticarcillin treated patients despite a piperacillin sodium content which is only 20% that of ticarcillin. Nevertheless, the piperacillin plus amikacin regimen must be regarded as highly effective and minimally toxic.
7. Moxalactam is a cephalosporin-like compound which, unlike currently marketed cephalosporins, has activity against Pseudomonas aeruginosa. A combination of moxalactam with amikacin has not only a broad spectrum but includes synergistic activity against P. aeruginosa, K. pneumoniae, E. coli and Staphylococcus aureus. As a result, moxalactam plus amikacin is now being compared to ticarcillin plus amikacin in a prospective, randomized double blind trial for febrile neutropenic cancer patients. Fifty-two patients have been treated but at least 100 patient-trials will be necessary to complete the evaluation.
8. Because of the potential for aminoglycoside nephrotoxicity, albeit minimal when these agents are used with ticarcillin or other β -lactam antibiotics, a comparative evaluation of approaches to aminoglycoside dosing has been established. One group of 60 patients received amikacin plus ticarcillin using standard "package insert" dosing instructions while another 60 patients are receiving amikacin (plus ticarcillin) dosed by a pharmacokinetic approach utilizing a hand-held programmable calculator. To date, the calculator accurately assigns the proper dose and interval to achieve a peak amikacin blood level of $25\mu\text{g/ml}$ and a trough level of $8\mu\text{g/ml}$. The dose required proves to be substantially greater than the "package insert" dose in younger patients and subsequently less in older patients. This may help to explain why older patients are more prone to nephrotoxicity when treated with aminoglycosides utilizing the standard procedures for calculating the appropriate dose.
9. The next empiric antibiotic evaluation will be a prospective, randomized comparison of three two-drug combinations: 1) ticarcillin plus amikacin (control), 2) cefatoxime plus amikacin, and 3) azlocillin plus amikacin. This protocol seeks to determine if the penicillin, azlocillin (which unlike ticarcil-

lin has activity against Klebsiella pneumoniae and increased activity against P. aeruginosa), or the cephalosporin, cefatoxime (which unlike ticarcillin has activity against Klebsiella pneumoniae and increased activity against S. aureus) will be superior to ticarcillin (plus amikacin) and concurrently produce less toxicity (especially hypokalemia). This study will also examine the issues of 1) length of antibiotic therapy required and 2) development of fungal superinfection.

Publications:

1. Bender, J.F., Fortner, C.L., Schimpff, S.C., Grove, W.R., Hahn, D.M., Love, L.J. and Wiernik, P.H.: Comparative auditory toxicity of aminoglycoside antibiotics in leukopenic patients: a randomized prospective trial. Am.J. Hosp. Pharm. 36:1083-1087, 1979.
2. Moody, M.R., Young, V.M., Schimpff, S.C., Purvis, D.S. and Wiernik, P.H.: In vitro antibacterial activity of four aminoglycosides and two semisynthetic penicillins on fresh clinical isolates from cancer patients. Europ. J. Cancer 15:23-33, 1979.
3. Murillo, J., Standiford, H.C., Schimpff, S.C. and Tatem, B.A.: Gentamicin and ticarcillin serum levels. JAMA 241:2401-2403, 1979.
4. Wade, J.C. and Schimpff, S.C.: Antibiotic therapy for febrile granulocytopenic patients. In Klastersky, J. and Staquet, M. (Eds.): Supportive Care in Cancer Patients. I, New York, Raven Press, 1980 (in press)
5. Love, L.J., Schimpff, S.C., Schiffer, C.A. and Wiernik, P.H.: Improved prognosis for granulocytopenic patients with gram-negative bacteremia. Am. J. Med. 68:643-648, 1980.
6. EORTC International Antimicrobial Therapy Project Group: Empirical therapy in febrile granulocytopenic patients. In Morris-Jones, P.H. (Ed.): Topics in Paediatrics, Haematology and Oncology, Kent, England, Pitman Medical Publishing Co., Ltd., 1979, pp. 113-124.
7. Schimpff, S.C.: Nephrotoxic potential of the cephalosporin-aminoglycoside combination. (Letter) Cancer Treat. Rep. 63:11-12, 1979.
8. Wade, J.C., Schimpff, S.C., Newman, K.A., Fortner, C.L., Moody, M.R., Young, V.M. and Wiernik, P.H.: Potential of mezlocillin for empiric single agent therapy in febrile granulocytopenic cancer patients. Antimicrob. Ag. Chemother. (in press) 1980.
9. Wade, J.C., Schimpff, S.C. and Wiernik, P.H.: Antibiotic combination-associated nephrotoxicity in granulocytopenic patients with cancer. Ann. Intern. Med. (in press) 1980

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06913-07 COB
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PERIOD COVERED October 1, 1979 through September 30, 1980
TITLE OF PROJECT (80 characters or less) Bronchial Brushing for Pulmonary Infiltrates in Patients with Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Joseph Aisner, M.D.	Head, Section of Medical Oncology	COB NCI
Others: Stephen C. Schimpff, M.D.	Head, Section of Infection Research	COB NCI
James C. Wade, M.D.	Senior Investigator	COB NCI
Viola M. Young, Ph.D.	Head, Section of Microbiology Research	COB NCI
Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB NCI

COOPERATING UNITS (if any) Pathology and Radiology Departments, Pulmonary Section, Department of Medicine, University of Maryland Hospital

LAB/BRANCH Clinical Oncology Branch
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SECTION Section of Infection Research
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INSTITUTE AND LOCATION NCI, NIH	Baltimore, Maryland 21201
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TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
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CHECK APPROPRIATE BOX(ES)		
☒ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☐ (c) NEITHER
☐ (a1) MINORS ☐ (a2) INTERVIEWS		

SUMMARY OF WORK (200 words or less - underline keywords) The myelosuppressed patient with cancer may develop pneumonias which are difficult to diagnose. Thus, aggressive diagnostic procedures are warranted. This study utilized a step-wise, orderly method for diagnosing unexplained <u>pulmonary infiltrates</u> utilizing <u>transtracheal aspiration</u>, <u>transtracheal bronchial brushing</u>, <u>fibroptic bronchoscopy</u> or <u>percutaneous lung biopsies</u> when other methods have failed. Transtracheal aspiration led to diagnosis in about 20-30% of cases. Bronchial brushing yielded diagnostic information in about 30% of cases overall and in nearly 80% of those ultimately proven to have infectious problems. Thirty to 40% of cases are ultimately shown to have noninfectious etiologies such as metastases, interstitial fibrosis or subsegmental collapse. Among the infectious etiologies the diagnosis included a variety of micro-organisms: bacterial, protozoal, and fungal. Significant but non-fatal complications occurred and were acceptable risks for the patient population. There was no mortality associated with the procedures, and despite pancytopenia, bleeding was a problem in only 3 patients. This step-wise aggressive diagnostic procedure has proven to be clinically efficacious in 80% of the patients with ultimately documented pneumonias and is now considered standard therapy.
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The myelosuppressed cancer patient frequently develops infectious complications, particularly pneumonia. Such infections are a contributory factor in the demise of a large percentage of these patients. One of the reasons for the high mortality is the difficulty in establishing an etiologic agent which would allow for the provision of appropriate antimicrobial therapy. Since there is an established low yield of noninvasive culture techniques, more aggressive diagnostic procedures are warranted. In addition, myelosuppressed patients frequently carry gram-negative organisms and fungi in their naso-oropharynx which will contaminate expectorated sputum. Since such patients rarely produce sputum spontaneously, the purpose of this study is to establish a method for diagnosing unexplained pulmonary infiltrates when conventional methods such as sputum induction have been unsuccessful. The study utilizes transtracheal aspiration, selective bronchial brushing through a transtracheal approach, peroral fibroptic bronchoscopy and percutaneous lung biopsies when other methods have failed.

Transtracheal aspiration alone provided a diagnosis in approximately 30% of the cases. Twenty percent of patients were not considered clinically infected and had such problems as pulmonary metastases, interstitial fibrosis or subsegmental collapse. Among patients presumed clinically infected, nearly 60% are proven to have pulmonary infection and the majority are etiologically documented by the brushing technique. For some (20%) of the presumed infected patients, no etiology can be established by any method. Most of these patients are receiving broad spectrum antibiotics at the time of the procedure. Diagnosis established by the brushing procedure included pneumonias involving such organisms as Aspergillus, Mucorales, Torulopsis glabrata, Staphylococcus aureus, Mycobacterium tuberculosis, Pneumocystis carinii, as well as aerobic and anaerobic Streptococci. Significant but nonfatal complications included hemoptysis, pneumothorax, and cervical cellulitis. There was no mortality associated with this procedure. Despite pancytopenia, bleeding has been a significant problem in only 3 patients. This stepwise procedure for diagnosis is now considered standard therapy.

Proposed Course: Discontinue Investigation.

Infections with Candida sp, Torulopsis glabrata, Aspergillus sp and Mucorales are increasing as more patients survive their initial bacterial infections. Amphotericin B is the only established antibiotic of defined activity in patients that are granulocytopenic, yet the response rate is low. Recently, rifampin has been shown in vitro to be synergistic with amphotericin B against these fungal organisms. Animal data have substantiated these in vitro results, and the BCRP has therefore begun a prospective randomized trial of amphotericin B vs amphotericin B plus rifampin as initial therapy for proven or suspected fungal infections.

A) Early Therapy of Invasive Aspergillus Infections: Aspergillus infections in cancer patients are difficult to diagnose and such diagnoses are frequently made at necropsy. Earlier therapy has been previously documented at our center to provide for better response, and earlier therapy is usually made by more aggressive diagnostic procedures. Not all patients, however, can undergo such invasive procedures so that other methods of identifying the infected or potentially infected patients are still needed. A review of patients with acute nonlymphocytic leukemia revealed A. flavus on nose cultures is predictive for aspergillosis. In addition, there appears to be a relationship between sterile nose culture and aspergillosis with a suggestion of a relationship between carbenicillin usage, sterile nose cultures, and positive nose cultures for Aspergillus. These data may help identify patients at risk of aspergillosis, and help determine antifungal therapy when invasive procedures are contraindicated.

B) Evaluation of Rifampin in Combination with Amphotericin B: Infections with Candida sp, Torulopsis glabrata, Aspergillus sp and Mucorales are increasing as more patients survive their initial bacterial infections. Not surprisingly therefore, the increase in systemic mycoses is associated with increased use of systemic broad-spectrum antibacterial antibiotics. Although other drugs have been introduced for the treatment of systemic mycoses, amphotericin B remains the primary antibiotic of defined efficacy for treatment of systemic mycoses in myelosuppressed patients. Recently, rifampin has been shown in vitro to be synergistic with amphotericin B against these yeast and fungal organisms. In addition, these studies have been confirmed by animal experimentation. The BCRP has therefore begun a prospective randomized trial of amphotericin vs amphotericin plus rifampin for initial therapy for proven or suspected fungal infections. Fifty patients have been randomized to date, 26 to amphotericin B and 24 to amphotericin B plus rifampin: after separation into the various types of fungal infections (several Candida sp, Torulopsis glabrata, Aspergillus sp and Mucorales, there are too few patients in any individual category to show any difference should a difference exist.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06951-04 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Infection Research in Oncology		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: S.C. Schimpff Other: J.C. Wade J. Aisner K.A. Newman P.H. Wiernik V.M. Young C.A. Schiffer	Head, Section of Infection Research Clinical Associate Head, Section of Medical Oncology Infection Control Nurse Chief, Clinical Oncology Branch Head, Research Microbiology Section Head, Section of Cell Component Therapy	COB NCI COB NCI COB NCI COB NCI COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) Division of Infectious Diseases, University of Maryland School of Medicine		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Infection Research		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.25	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Infection is the leading cause of morbidity and mortality in patients with acute leukemia and a major cause in other types of advanced malignancies. The aim of this program is an <u>integration of research, education, and patient care with regard to the infections arising in patients with cancer.</u> The research areas include <u>infection epidemiology, diagnosis, prevention and treatment.</u> Education includes the teaching of BCRP clinical associates the fundamental problems and techniques for infection management, teaching the nursing staff basic prevention and treatment modalities, and teaching the University of Maryland Infectious Disease fellows and students concepts of infection control in the tumor patient. A program of continuing education through seminars, symposia and publications serves the medical community at large. Patient care includes patient education in infection prevention in addition to infection diagnosis, prevention and management.		

The frequency and severity of infection in patients with advanced cancer necessitates an ongoing program of 1) infection research, 2) education and 3) patient care. The aims of this program are to integrate these three areas into the ongoing clinical and laboratory programs at the BCRP.

Research areas include infection epidemiology, diagnosis, prevention and therapy (see #6264, #6266, #6950 and #6217).

Education includes the training of BCRP clinical associates, University of Maryland Infectious Disease fellows, and Department of Medicine rotating residents in the management approaches necessary for optimum care of the infected or potentially infected cancer patient. This is done through daily work rounds plus informal discussions. Nursing education, done largely by the infection control nurse, is accomplished by twice monthly teaching conferences plus informal patient management discussions.

An integrated team approach has been developed, based around twice weekly formal infection rounds, and includes active participation by physicians from the Infection Research Section and the Cell Support Program, the infection control nurse, microbiologist, pharmacist, and attending physicians. This approach ensures thorough discussion of the various management approaches for each patient while reviewing the requirements of protocol studies.

A full day symposium was held for 360 physicians at the BCRP in January, 1980. The eight member panel updated the current approaches to diagnosis, prevention and treatment of infection in the compromised host with emphasis upon the patient with cancer.

Publications:

1. Schimpff, S.C.: Infection in the compromised host. In Bennett, J.E. (Ed.): Principles and Practices of Infectious Diseases. New York, John Wiley and Sons, Inc., Publ., 1979, pp. 2257-2262.
2. Schimpff, S.C.: Infections in patients with acute leukemia with emphasis on infections associated with granulocytopenia. In Bennett, J.E. (Ed.): Principles and Practices of Infectious Diseases. New York, John Wiley and Sons, Publ., 1979, pp. 2263-2278.
3. Schimpff, S.C.: Hodgkin's disease. In Bennett, J.E. (Ed.): Principles and Practices of Infectious Diseases. New York, John Wiley and Sons, Publ., 1979, pp. 2278-2284.
4. Schimpff, S.C.: Infection prevention during granulocytopenia. In Remington, J.S. and Swartz, M.N. (Eds.): Current Clinical Topics in Infectious Diseases. New York, McGraw-Hill Book Company, 1980, pp. 85-106.
5. Schimpff, S.C. and Wiernik, P.H.: Infection in cancer patients (predisposing factors, sites, organisms and general approach to diagnosis). In Silver, R.T. (Ed.): Topics in Cancer. New York, Physician Programs, Inc., 1980 (in press)

6. Schimpff, S.C. and Wiernik, P.H.: Therapy of infection in granulocytopenic patients. In Silver, R.T. (Ed.): Topics in Cancer. New York, Physician Programs, Inc., 1980 (in press)
7. Schimpff, S.C. and Wiernik, P.H.: Infections in the patient with cellular immune dysfunction. In Silver, R.T. (Ed.): Topics in Cancer. New York, Physician Programs, Inc., 1980 (in press)
8. Schimpff, S.C. and Wiernik, P.H.: Infection prevention in patients with cancer. In Silver, R.T. (Ed.): Topics in Cancer. New York, Physician Programs, Inc., 1980 (in press)

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 CM 06988-03 COB
PERIOD COVERED October 1, 1979 through September 30, 1980		
TITLE OF PROJECT (80 characters or less) Radiation-Related Thyroid Dysfunction in Hodgkin's Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: S.C. Schimpff Other: P.H. Wiernik P. Salvatore	Head, Section of Infection Research Chief, Clinical Oncology Branch Secretary, Section of Infection Research	COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) J.G. Wiswell, Section of Endocrinology, Department of Internal Medicine, University of Maryland School of Medicine		
LAB/BRANCH Clinical Oncology Branch		
SECTION Section of Infection Research		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 0.125	PROFESSIONAL: 0.062	OTHER: 0.062
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Late complications</u> in the treatment of <u>Hodgkin's disease</u> with radiation therapy are increasingly being recognized. <u>Thyroid dysfunction</u> following <u>irradiation</u> is one such complication which has not been widely recognized, probably because of the subtle clinical nature of minor thyroid hormone imbalance. In addition, it has long been thought that the thyroid was relatively resistant to radiation therapy. Two hundred thirty-two patients with Hodgkin's disease followed at BCRP have been evaluated for post-therapy thyroid dysfunction. Forty-one percent of those with mantle radiation therapy had an elevated TSH, but normal T₄, but an additional 25% had an elevated TSH plus a depressed T₄. Thus, 66% of 169 patients had evidence of thyroid dysfunction following mantle radiation therapy. Since thyroid hormone replacement is simple, inexpensive and usually without major side effects if properly monitored, it is reasonable in light of the foregoing to place all patients with an elevated TSH on thyroid replacement therapy whether or not they are chemically hypothyroid.		

Two hundred fourteen patients with Hodgkin's disease returning to the Baltimore Cancer Research Program for routine clinic visits had a serum sample obtained for thyroid function tests. Some patients had multiple serial serum samples taken over many months. Serum thyroxine (T_4), T_3 resin uptake by thyroxine binding globulin, the effective thyroxine ratio and the serum thyroid stimulating hormone (TSH) were measured. Patients with stages I-III A receiving radiation therapy or radiation plus adjuvant chemotherapy and patients with stages IIIB-IV received chemotherapy alone. Radiation therapy was delivered from a cobalt 60 source with midplane dose of approximately 4000 rads given as 200 rads per day, five days per week for four weeks weighted to give two-thirds of the dose from the anterior port and one-third through the posterior port.

Major Findings:

Of 169 patients who received mantle radiation therapy, 69 (or 41%) were found to have an elevated TSH but normal thyroid hormone level. An additional 43 patients (25%) had an elevated TSH plus an abnormally low T_4 . Combined, 112 of 169 patients (66%) had evidence of thyroid dysfunction following mantle radiation therapy. Among the 45 patients who had not received mantle radiation therapy, 3 (7%) had an elevated TSH but none had depressed thyroid hormone levels.

With an incidence of 25% of patients developing chemical hypothyroidism within a few years after radiation therapy, it is clear that this is a major complication of irradiation for Hodgkin's disease. All such patients should therefore have TSH determinations done every six months for the first five years following irradiation with determination of thyroxine levels should the TSH be elevated. Replacement therapy with sodium levothyroxine will usually be sufficient. For those patients with elevations of TSH alone, it is appropriate to begin replacement therapy for this state of compensated hypothyroidism. Patients may benefit symptomatically, the lessened production of TSH will prevent the rare occurrence of sella turcica damage, and, since elevated TSH in the presence of radiation damaged thyroid tissue is known to be carcinogenic, TSH suppression may lessen the potential for later development of thyroid carcinoma.

Publications:

1. Schimpff, S.C.: Well differentiated thyroid carcinoma: epidemiology, etiology and treatment. Amer. J. Med. Sci. 278:100-114, 1979.
2. Schimpff, S.C.: Carcinoma of the thyroid. In Aisner, J. and Chang, P. (Eds.): Advances in Cancer Treatment Research. The Netherlands, Martinus Nijhoff, Publ., 1980, pp. 88-102.
3. Schimpff, S.C.: Hormonally active tumors. In Lichtman, M.A. and Wiernik, P.H. (Eds.): The Science and Practice of Clinical Medicine. New York, Grune and Stratton, 1980 (in press)
4. Schimpff, S.C., Diggs, C.H., Wiswell, J.G., Salvatore, P.C. and Wiernik, P.H.: Radiation-related thyroid dysfunction: implications for the treatment of Hodgkin's disease. Ann. Intern. Med. 92:91-98, 1980.

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Microbiological Services for Clinical and Research Studies of Cancer Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Viola Mae Young, Ph.D.	Head, Research Microbiology Section	COB NCI
Others: Gloria M. Thomas	Supervisor	COB NCI
Norman Friedman	Microbiologist	COB NCI
Gail B. Marriott	Lab. Scientist	COB NCI
William R. Miller	Lab. Scientist	COB NCI
Debra B. Schaum	Lab. Scientist	COB NCI
Eric N. Talley	Lab. Technician	COB NCI
Carol A. White	Lab. Scientist	COB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

Research Microbiology Section

INSTITUTE AND LOCATION

NCI, NIH Baltimore, Md. 21201

TOTAL MANYEARS:

2

PROFESSIONAL:

1.50

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

As Infection is a leading cause of morbidity and mortality in cancer patients with severe granulocytopenia and other damage to host defenses, this project was designed to detect the presence of infectious agents in these patients prior to and during infection to provide a sound base for antibiotic therapy. It is also designed to detect carriers and/or "shedders" of potential pathogens or highly resistant strains so that they can be isolated from the high risk patients. In addition it affords microbiological data for a number of protocols which involve infection in cancer patients. To provide such data all patient samples are studied intensively and all of the microorganisms recovered are identified as to genus and species. Surveillance cultures taken from high risk patients provide data on patient flora at admission and subsequent acquisition during the hospital stay to alert the physician to their presence and antibiotic susceptibility prior to infection.

Project Description:

Infection causes much of the morbidity in patients with acute leukemia and may result in death before adequate anticancer therapy can be given. The purpose of this project is to provide high quality microbiologic study of samples collected from the cancer patients in order to prevent and/or to optimally treat such infection. The studies of the samples serves several purposes: 1) to recover bacteria, mycoplasma or fungi from infectious processes and determine their minimum inhibitory concentration (MIC) to a selected battery of antibiotics in order to allow selection of appropriate antibiotic therapy; 2) surveillance culture data allows isolation of patients who carry pathogenic or highly resistant organisms and provides a means of monitoring patient acquisition of microorganisms from the hospital personnel or environment; 3) the data provides information required by protocols of the Infections Research Section and a basis for reviews and investigations of microorganisms and the infections they cause in cancer patients.

These studies are all performed by personnel of the Microbiology Section of the Baltimore Cancer Research Program. Identification schema, reporting systems, contracts, and work flow patterns have been developed. The MIC 2000 has been evaluated as a tool for simultaneously performing MICs and inoculating a group of 20 to 24 biochemicals for rapid speciation of bacteria. Personnel have now been trained in the use of this equipment and it is in routine use. Responsive interaction with patient care physicians and the Infections Research Section and participation in Infections Rounds continues.

Microbiologic support for a number of protocols and projects is supplied by this project. Among these are several projects with the Infections Research Section (06264, 06217, 06951, 06266, 06950, 06913). These microbiologic studies have provided data essential to patient care, further information concerning resistant diphtheroids, continued data on the prevalence of gram positive microflora or fungi in certain groups of patients and on prevalence of bacteremias caused by Staphylococcus epidermidis. It is anticipated that such microbiologic studies will be continued.

Publications:

1. Love, L.J., Schimpff, S.C., Hahn, D.M., Young, V.M., Standiford, H.C., Bender, J.F., Fortner, C.L. and Wiernik, P.H. Randomized trial of empiric antibiotic therapy with ticarcillin in combination with gentamicin, amikacin or netilmicin in febrile patients with granulocytopenia and cancer. *Am. J. of Med.* 66: 603-609, 1979.
2. Bender, J.F.*, Schimpff, S.C., Young, V.M., Fortner, C.L., Brouillet, M.D., Love, L.J. and Wiernik, P.H. Role of vancomycin as a component of oral nonabsorbable antibiotics for microbial suppression in leukemic patients. *Antimicrob. Ag. & Chemother.* 15: 455-460, 1979.

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Computerized Microbiology Reporting and Data Retrieval Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Viola Mae Young, Ph.D.

Head, Research Microbiology
Section

COB NCI

Others: Joann Gustafson

Computer Specialist

COB NCI

COOPERATING UNITS (if any)

None

LABORATORY

Clinical Oncology Branch

SECTION

Research Microbiology Section

INSTITUTE AND LOCATION

NCI, NIH

Baltimore, Md. 21201

TOTAL MAN-YEARS:

.66

PROFESSIONAL:

.33

OTHER:

.33

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☐ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project provides a computerized system for reporting the results of microbiologic studies and storage of the results thus accrued for research purposes. Data from the final reports are temporarily accumulated in a commercial computer and at regular intervals transferred in batches to a computer center where it is processed through error detection and quality control systems. A final report is generated and reviewed and errors are corrected. Individual patient data are regularly summarized as new data are entered and these summary reports are placed in the patient's charts and copies are filed in a central location. Transfer of the present microbiologic computer systems to the PROMIS system (See Project # Z01 CM 0625911 COB) is under evaluation as the Computer Center in current use will close.

Project Description:

Final reports of all microbiologic data are sent from a computer terminal in the laboratory office to a commercial computer and then transferred in batches at regular intervals to a computer center. There the data are processed through error detection systems to ensure accuracy prior to permanent storage. Final reports are generated and reviewed for accuracy. All errors are reviewed and corrected and new reports generated and the data stored. At regular intervals individual patient data are summarized as new data and are entered, and the summary then placed in the patient's chart. Copies of these summary reports are filed in a central location.

All microbiologic data generated since 1968 from patients at the Baltimore Cancer Research Program have been stored. As the data base accrued since that time is very large and the magnetic disks were filled, data prior to 1976 has been removed to tapes for further use and storage. The closure of the Computer Center in current use is imminent and transfer of the present microbiologic systems to the PROMIS system (see Project # Z01 CM 0625911 COB) is under evaluation. Recent data retrievals covered all positive blood cultures since 1968, and Staphylococcus epidermidis and Pseudomonas aeruginosa recovered from sources which indicate probable infections. Publications based on these printouts are in preparation.

PROJECT NUMBER (Do NOT use this space)	HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	ZO1 CM 06219-11 COB												
PERIOD COVERED October 1, 1979 to September 30, 1980														
TITLE OF PROJECT (50 characters or less) Studies on Cancer Patient Microbial Flora: Acquisition, Colonization, & Infection														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Viola Mae Young, Ph.D.</td> <td style="width: 33%;">Head, Research Microbiology Section</td> <td style="width: 34%;">COB NCI</td> </tr> <tr> <td>Others: Marcia R. Moody, Ph.D.</td> <td>Research Microbiologist</td> <td>COB NCI</td> </tr> <tr> <td>Stephen C. Schimpff, M.D.</td> <td>Head, Section of Infection Research</td> <td>COB NCI</td> </tr> <tr> <td>James C. Wade, M.D.</td> <td>Clinical Associate</td> <td>COB NCI</td> </tr> </table>			PI: Viola Mae Young, Ph.D.	Head, Research Microbiology Section	COB NCI	Others: Marcia R. Moody, Ph.D.	Research Microbiologist	COB NCI	Stephen C. Schimpff, M.D.	Head, Section of Infection Research	COB NCI	James C. Wade, M.D.	Clinical Associate	COB NCI
PI: Viola Mae Young, Ph.D.	Head, Research Microbiology Section	COB NCI												
Others: Marcia R. Moody, Ph.D.	Research Microbiologist	COB NCI												
Stephen C. Schimpff, M.D.	Head, Section of Infection Research	COB NCI												
James C. Wade, M.D.	Clinical Associate	COB NCI												
COOPERATING UNITS (if any) None														
LAB/BRANCH Clinical Oncology Branch														
SECTION Research Microbiology Section														
INSTITUTE AND LOCATION NCI, NIH Baltimore, Md. 21201														
TOTAL MANYEARS: 2	PROFESSIONAL: 1.50	OTHER: .50												
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER														
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to monitor the patients' microbial flora which may give rise to infection, to determine sources of hospital acquired flora when possible, and to study characteristics of unusual organisms of clinical significance. Such organisms may be subspeciated by phage typing, serotyping or biotyping to allow determination of spread. A review of cultures, particularly blood cultures, revealed that during the last year gram positive cocci outnumbered gram negative bacilli as a cause of bacteremias. <u>Staphylococcus epidermidis</u> and streptococci both increased significantly in numbers. Antibiotic resistant coryneform bacteria are still encountered in the patients and further studies have been carried out to evaluate their characteristics. Nasal surveys for filamentous fungi confirmed the presence of the organisms in the air as has been shown in air samples (Project No. ZO1 CM 06938-11 COB). <u>Staphylococcus aureus</u> surveys of personnel showed the carrier rate to be sharply decreased.														

Project Description:

Opportunistic microorganisms that are considered "normal" microflora in a normal human host, are the chief cause of infection in cancer patients whose host defenses are impaired. This project is designed to monitor the cancer patients' flora, to determine sources of such flora, and to study unusual microorganisms that are recovered.

It was observed that there has been a change in the microorganisms recovered from leukemic patients toward higher recoveries of gram positive cocci. Slightly over half of the organisms grown from blood in 1979 were gram positive cocci (Staphylococcus epidermidis 20.8%, S. aureus 11.8% and streptococci 18.2%). During an earlier period (1974-1976) S. epidermidis comprised 10.2%, S. aureus 12.2%, and streptococci 6.2% of the positive blood cultures. In contrast Pseudomonas aeruginosa (at one time the chief cause of infectious death) decreased from 17.5 to 6.4% and Escherichia coli from 18.5 to 11.2%. S. epidermidis strains recovered from blood and other infectious processes were sent to a phage typing center where it was determined that the increase in numbers of such infections were not due to the spread of a single strain. Only minor shifts were noted in other flora. During the previous study the leukemia patients received the combination of gentamicin, vancomycin, and nystatin as oral prophylactic antibiotics. During 1979 vancomycin was dropped from the prophylactic combination and an additional group of patients received trimethoprim-sulfamethoxazole with nystatin prophylactically. The absence of vancomycin undoubtedly made a major contribution to the increase in S. epidermidis recovery rates.

The coryneform bacterium that is susceptible to vancomycin and occasionally to rifampin, but to none of the other antibiotics tested, is still occasionally encountered in the patient population. It was recovered from samples of approximately six patients last year. All of the strains thus far recovered have been run through a series of 50 biochemical reactions by means of 50L API strips and the results are under comparison with those obtained with conventional techniques.

The addition of surveillance nose cultures for filamentous fungi reported last year have been continued, as infections caused by these organisms remain a problem in the cancer patients. These cultures reveal that considerable variations in the number of positive cultures occur over time. For example, in January 1980, 8.3% of the 157 fungal nose cultures grew filamentous fungi, all from different patients, whereas in February 1980, 17.7% of the total 141 nose cultures for fungi yielded these organisms, including 9 Aspergillus flavus from 8 patients.

The last survey of hospital personnel to determine Staphylococcus aureus carriers revealed that the carriage rate had dropped to a low of approximately 6%.

Publications:

1. Schimpff, S.C. and Young, V.M. Epidemiology and prevention of infection in the compromised host, "Clinical approach to infection in the immunocompromised host", R.H. Rubin and L.S. Young, Plenum Publishing Corp. N.Y. In Press, 1980.
2. Young, V.M., Moody, M.R. and Morris, M.J. Phase variation in the genus *Serratia*. J. Med. Microbiol. In Press, 1980.
3. Young, V.M., Moody, M.R. and Morris M.J. Distribution of *Serratia marcescens* serotypes in cancer patients. J. Med. Microbiol. In Press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 CM 06223-11 COB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies on *Pseudomonas aeruginosa* in a Cancer Research Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Marcia R. Moody, Ph.D.

Research Microbiologist

COB NCI

Others: Viola Mae Young, Ph.D.

Head, Research Microbiology
Section

COB NCI

Norman R. Friedman

Microbiologist

COB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Oncology Branch

SECTION

Research Microbiology Section

INSTITUTE AND LOCATION

NCI, NIH

Baltimore, Md. 21201

TOTAL MANYEARS:

.66

PROFESSIONAL:

.50

OTHER:

.16

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Pseudomonas aeruginosa is one of the chief causative agents of infection in severely granulocytopenic patients with acute leukemia and other malignancies. The prime objective of the study has been to monitor its epidemiology in cancer patients, to study its host-parasite interaction, and its antibiotic susceptibilities. Recovery rates are higher than last year. Serotype 12, (two patient recoveries in 10 years was recovered from four patients. Serotypes of *P. aeruginosa* varied in their ability to activate the alternate complement pathway in nonimmune serum. Certain serotypes completely consumed complement by 1) the alternate pathway or 2) the classical pathway; other serotypes incompletely de complemented this serum via the classical pathway. Recovery of nonfermentative gram negative bacilli from patients as well as their environment has increased; the majority include bacterial species that had been less frequently encountered in this patient population during the last ten years. *P. paucimobilis*, a recently described species, was also recovered. The predominant species among fluorescent pseudomonads isolated from surface waters, pools, etc.

Project Description:

Pseudomonas aeruginosa remains a frequent cause of infection in cancer patients and the prevention of infections by this ubiquitous organism continues to be an important goal. This project monitors its incidence, epidemiology, and host-parasite interactions. The role of other nonfermentative gram negative bacilli in these patients and their environs is also evaluated on a continuous basis.

Serotyping of all strains of P. aeruginosa recovered is still performed on a routine basis. The recovery of P. aeruginosa has increased when compared with its frequency last year but its incidence is still not as high as in previous years. Serotype 12, a serotype rarely recovered from this patient population (2 patients in ten years), was recovered from four patients; there was no evidence of transmission between patients. P. aeruginosa continues to be infrequently isolated from the hospital environment. Serotype variability in the activation of the alternate complement pathway was investigated to determine if this intrinsic ability or inability of a serotype to evade opsonization via alternate pathway activation in a pre-specific antibody state could be associated with the invasive capabilities of certain serotypes. Normal pooled human serum was completely de complemented 1) via the alternate pathway by serotypes 3,4,9,10, and 11, and 2) via the classical pathway by serotypes 1,6,12, and 13. The third group, which included 2,5,7, and 8, consumed about half the complement in this normal serum via the classical pathway. Strains from the first group were rarely recovered from the blood but those strains that were recovered from this source, activated complement via the classical pathway. Most strains from the second group were recovered from wounds, lesions, or blood. The strains from the third group were most often recovered from the blood. In contrast to some of the strains from the first group, those strains isolated from sources other than blood; i.e., the rectum and urine, also inefficiently consumed complement via the classical pathway. Further studies are in progress.

Recovery of nonfermentative gram negative bacilli from patients as well as their environment has increased. The incidence of P. maltophilia has remained constant, but the recovery of bacilli less frequently encountered in this patient population, such as Moraxella, Bordetella, Acinetobacter, and non-saccharolytic pseudomonads, has risen. The incidence of fluorescent pseudomonads other than P. aeruginosa has decreased. P. paucimobilis, a recently described species, was isolated from sputa.

Water is considered a common source of P. aeruginosa contamination; in some instances the deciding factor in identification of Pseudomonas strains recovered from water has been the ability of the isolated organism to produce fluorescein. More complete characterization of fluorescent pseudomonads isolated from surface waters and pools inside and outside the USA has shown that P. putida appears to be the predominant species; this species was also consistently isolated from the inner toilet rims at the BCRP when located at the U. of Md. hospital as well as the PHS hospital.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06254-11 COB									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less) Research Activities Directed Toward Improvement of Microbiology Laboratory Techniques											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT											
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> PI: Viola Mae Young, Ph.D. </td> <td style="width: 50%; vertical-align: top;"> Head, Research Microbiology Section </td> <td style="width: 10%; text-align: right; vertical-align: top;"> COB NCI </td> </tr> <tr> <td colspan="3" style="height: 10px;"></td> </tr> <tr> <td style="vertical-align: top;"> Others: Marcia R. Moody, Ph.D. Gloria M. Thomas Michael T. Hargadon </td> <td style="vertical-align: top;"> Research Microbiology Supervisor Microbiologist </td> <td style="text-align: right; vertical-align: top;"> COB NCI COB NCI COB NCI </td> </tr> </table>			PI: Viola Mae Young, Ph.D.	Head, Research Microbiology Section	COB NCI				Others: Marcia R. Moody, Ph.D. Gloria M. Thomas Michael T. Hargadon	Research Microbiology Supervisor Microbiologist	COB NCI COB NCI COB NCI
PI: Viola Mae Young, Ph.D.	Head, Research Microbiology Section	COB NCI									
Others: Marcia R. Moody, Ph.D. Gloria M. Thomas Michael T. Hargadon	Research Microbiology Supervisor Microbiologist	COB NCI COB NCI COB NCI									
COOPERATING UNITS (if any)											
LAB/BRANCH Clinical Oncology Branch											
SECTION Research Microbiology Section											
INSTITUTE AND LOCATION NCI, NIH Baltimore, Md. 21201											
TOTAL MANYEARS: 1.33	PROFESSIONAL: 1	OTHER: .33									
CHECK APPROPRIATE BOX(ES)											
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER											
☐ (a1) MINORS ☐ (a2) INTERVIEWS											
SUMMARY OF WORK (200 words or less - underline keywords)											
The project provides for development of methodology and its implementation in response to the needs of patient care and/or the microbiology laboratory. Two tests for the detection of <u>Clostridium difficile</u> in patients with diarrhea have been evaluated and set up. API systems have been tested and are being used for yeast and anaerobe identifications. The API system for identification of <u>Enterobacteriaceae</u> was compared to the MIC 2000 identification system with similar results. An immuno diffusion kit for detection of antibodies against <u>Aspergillus</u> is under evaluation and FA techniques for identification of <u>Legionella pneumophila</u>, <u>Listeria</u>, and <u>Candida albicans</u> readied for use.											

Project Description:

This project is maintained to develop, adopt, and evaluate new methodologies in response to new or changing needs in the patient care and laboratory areas.

It has been reported that Clostridium difficile causes diarrhea in patients in whom overgrowth of this organism in the intestine occurs. In the BCRP setting wherein oral prophylactic antibiotics are given to suppress facultative anaerobic flora (but not anaerobes) the possibility exists of overgrowth by C. difficile. Two methods have been investigated to detect this organism. 1) Growth of the organism in 0.2% paracresol in BHI, and 2) Detection of toxin produced by C. difficile in WI 38 tissue cells.

Further development of anaerobic techniques is in progress. These include the use of API anaerobe strips for identification of anaerobes and preliminary work with gas chromatography for the same purpose.

The API system has also been introduced and evaluated for the identification of yeasts. All yeasts that have not been shown by preliminary work to develop chlamydospores (Candida albicans) are now identified by this procedure.

An immunodiffusion kit for detection of antibodies against Aspergillus and other filamentous fungi is being evaluated. Preliminary tests with several patient antisera (known to have had fungal infections) have been promising. Further evaluations are in progress.

A one week comparison was made between the MIC 2000 utilized in the Microbiology Laboratory and the API E 20 System for identification of gram negative bacilli recovered from patient samples. Results were entirely comparable with the exception that identification of one organism was made by the MIC 2000 and not the API E20.

FA antisera for Legionnaire's, Listeria, Meningococcus (polyvalent), streptococcus Group A, certain E. coli serotypes, Candida albicans and other yeasts have been obtained. The Legionnaire's, Listeria, and Candida FA antisera have been evaluated and the tests are ready for use. The others are under evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06938-06 COB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Environmental Studies to Prevent Hospital Acquisition of Potentially Dangerous Microflora		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Viola Mae Young, Ph.D. Others: Norman R. Friedman Marcia R. Moody, Ph.D. Stephen C. Schimpff, M.D. Kathy Newman	Head, Research Microbiology Section Microbiologist Research Microbiologist Head, Section of Infection Research Infection Control Nurse	 COB NCI COB NCI COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Research Microbiology Section		
INSTITUTE AND LOCATION NCI, NIH Baltimore, Md. 21201		
TOTAL MANYEARS: .75	PROFESSIONAL: .25	OTHER: .50
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
The purpose of this continuing study is to monitor the hospital environment and protective isolation rooms for sources of microorganisms which can, given the opportunity, colonize and eventually infect the host-impaired cancer patient. As such sources are detected; procedures are instituted to prevent recurrence. Examples of problem areas detected last year are; 1)certain contaminated foods, 2) contaminated ice from hospital kitchen, 3) contaminated Coca cola, 4) improperly cleaned ICU rooms and 5) contaminated denture cups.		

Project Description:

Many of the infections that occur in cancer patients are caused by microorganisms that are acquired by the patient from the hospital environment. The purpose of this continuing project is to monitor the patient's physical environment, the products used in their care, and the personnel involved in their care, in order to detect and eliminate, insofar as possible, the sources of such organisms. Regularly scheduled cultures of laminar air flow rooms are performed once or twice weekly to monitor protective procedures; general personnel studies are carried out twice per year and persons in contact with patients in laminar air flow rooms are cultured on a weekly basis. Isolation rooms are monitored for the presence of antibiotic resistant microorganisms following cleaning procedures when the isolated individual leaves the room, and weekly cultures of food, air, water, room surfaces and, less frequently, pharmaceuticals for the LAF rooms, are done.

Cultures of food during the past year were not as consistently free from bacteria as previously. Penicillium and Cladosporium species were grown from baked potato, Rhodotorula and Cladosporium sp from mashed potato, Enterobacter agglomerans from custard, Group D streptococci from a casserole of ground beef and macaroni, and Aspergillus niger from veal parmigiana. Of the non-cooked food, several species of non-fermenting gram negative bacilli and Escherichia coli were recovered from a culture of toasted white bread; Coca cola regularly grew the yeast Rhodotorula and frequently Candida parapsilosis, and cultures of puffed rice grew Aspergillus flavus. Ice from the hospital kitchen regularly yielded Aspergillus niger when cultured. Such results allowed recommendations to be made for elimination of some foods and for care in handling other food items that were undoubtedly contaminated after having been cooked. Extensive studies were performed to evaluate supplies, procedures and personnel for new LAF rooms. Surfaces were relatively bacteria free except for occasional cultures of splash areas. Linen supplies were sterile with rare exceptions. Of the pharmaceuticals employed, only Nystatin suspension was contaminated (Bacillus sp.). The patients' gut flora was effectively suppressed, but gingival and nose cultures continued to harbor microorganisms. On the basis of the appearance of several microorganisms in the nose cultures that had not been recovered from earlier samples, the decision was reached to use an inhibitory nose cream in an attempt to suppress such flora.

Additional findings revealed by the environmental cultures were the ineffective cleaning of an LAF-ICU room following the departure of a patient who carried a resistant microorganism, recovery of fungi from the air of the LAF-ICU area, fungal contaminants of surfaces in the LAF-ICU dirty utility room, and the presence of a resistant Pseudomonas aeruginosa serotype 6 in an isolation room previously occupied by a patient who carried this resistant serotype 6 strain. Two patients had occupied the room since this carrier was present and at the time the organism was recovered.

Preliminary work in a collaborative study initiated by Dr. DePaola of the University of Maryland dental school revealed that denture cups and unclean dentures are often heavily contaminated by a variety of yeasts and gram negative bacilli. Microbiologic support will be provided for his effort to eliminate this source of oral contamination.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 06972-03 COB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Antibiotic Studies: Evaluation of New Drugs and Their Characteristics		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Marcia R. Moody, Ph.D. Others: Viola Mae Young, Ph.D. Stephen C. Schimpff, M.D. Gail L. Tillman	Research Microbiologist Head, Research Microbiology Section Head, Section of Infection Research Lab. Scientist	COB NCI COB NCI COB NCI COB NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Research Microbiology Section		
INSTITUTE AND LOCATION NCI, NIH Baltimore, Md. 21201		
TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) "Normal" microflora can cause serious infections in cancer patients and control of infections by prevention or elimination is essential. This study objective is to evaluate the <u>in vitro effectiveness of new antibiotics</u> against these bacteria and to monitor the <u>susceptibility trends</u> to antibiotics already in use. MK0787, an analogue of thienamycin, was a highly potent <u>inhibitor of enterobacteria</u>, <u>P. aeruginosa</u> and gram positive cocci, including those strains resistant to six <u>lactam antibiotics</u>; i.e., piperacillin, ticarcillin, carbenicillin, mezlocillin, azlocillin, and BAY K4999. Incidence of <u>mezlocillin-resistant strains</u> decreased to <u>pre-use levels</u> 1 year after <u>mezlocillin use discontinuation</u>. <u>Piperacillin</u> patterns remained the same. <u>Carbenicillin resistance</u> has <u>increased</u> among strains of <u>P. aeruginosa</u> and <u>E. coli</u>, as well as <u>ticarcillin resistance</u> among the latter strains and <u>Klebsiella</u> spp. The <u>Moxalactam-amikacin</u> combination exerted a <u>synergistic or partially synergistic</u> effect on about half of enterobacterial strains tested and about 90% of <u>P. aeruginosa</u> strains. <u>Amikacin-piperacillin</u> was also synergistic or partially so for most of the enterobacteria as well as <u>P. aeruginosa</u>. <u>Moxalactam-piperacillin</u> was usually synergistic for <u>P. aeruginosa</u> but not necessarily so for the enterobacteria.		

Project Description:

Bacteria that constitute normal flora in the host with intact defense mechanisms often become "opportunistic" pathogens in cancer patients with compromised defenses. As an adjunct to the antitumor therapy received by this "at risk" population, infections caused by these bacteria must be eliminated or prevented. Antimicrobial agents that are reported to have activity against a broad spectrum of microorganisms such as P. aeruginosa, Klebsiella spp., Serratia spp., and indole positive Proteus spp. are continually being developed. This study is designed to evaluate the in vitro effectiveness of new compounds as they become available against these infectious agents and to monitor changes in bacterial susceptibility patterns to antibiotics already in use. The in vitro antibacterial spectrum of MK0787, an analogue of thienamycin which is more chemically stable than the parent compound, against 101 strains of P. aeruginosa, 353 strains of enterobacteria, and 71 strains of gram positive cocci was determined by the broth dilution micromethod. All species were inhibited by 10 µg/ml or less. No inoculum effect was observed when the inoculum concentration was increased from 10^5 to 10^7 colony forming units/ml. This activity was compared with the in vitro spectra of piperacillin, mezlocillin, azlocillin, ticarcillin, carbenicillin, and BAY K 4999 against the same isolates. Some strains of all species except S. aureus were resistant to the latter agents. Of these six agents, BAY K4999 was the most effective. However, MK0787 was more than 100-fold more active than BAY K4999 against E. coli, E. cloacae, Serratia spp., and S. aureus, 16-fold more active against Klebsiella spp., and 8-fold more active against P. aeruginosa. The inhibitory activity was about the same for E. aerogenes and other Enterobacter spp., C. freundii, indole positive Proteus spp., and group D streptococci. BAY K 4999 was 4-fold more inhibitory for P. mirabilis.

Prior to the use of mezlocillin in an empiric antibiotic regimen, few of E. coli and Klebsiella strains were resistant to mezlocillin. During usage and just after, about one-third of the strains tested were resistant, whereas one year later the percentages of resistant strains had decreased to pre-use levels. In contrast, the number of resistant Serratia strains doubled during mezlocillin usage and remained at that level one year later. Also, although there was either no increase in resistant strains or no resistant strains among the bacteria tested one year later, there were shifts in the mezlocillin susceptibility patterns. The MIC₉₀₋₁₀₀ for most species except P. aeruginosa and indole positive Proteus spp., decreased to pre-use levels. The MIC₉₀ for P. aeruginosa increased from 50 µg/ml to 100 µg/ml and the level for indole positive Proteus spp. remained at 50 µg/ml.

The susceptibility patterns of bacteria tested this year with piperacillin, an antibiotic currently used upon occasion, did not differ appreciably from the patterns among bacteria tested 3 years ago. However, more strains of P. aeruginosa and E. coli were resistant to carbenicillin, an antibiotic not used for some time, than there were 3 years ago. There has also been an increase in Klebsiella and E. coli strains that are resistant to ticarcillin, an antibiotic in current use.

The in vitro activities of moxalactam and piperacillin when combined with amikacin or with each other against over 500 bacterial strains was determined by a checkerboard microdilution method. The MIC₉₅ for amikacin, piperacillin, and moxalactam alone was 2.5, 25, and 0.25 µg/ml (enterobacteria) or 32 µg/ml (P. aeruginosa), respectively. No antagonism occurred with any of the combinations.

When amikacin was used in any combination, its MIC's decreased to 0.63 $\mu\text{g/ml}$ or less. The use of the designation indifference or partial synergy among enterobacteria reflected that many moxalactam MIC's were 0.125 $\mu\text{g/ml}$, or a 2-fold decrease also resulted in an MIC of 0.125 $\mu\text{g/ml}$. Moxalactam-amikacin exerted a partial synergistic effect on 1/3 of the enterobacteria and a synergistic effect on 1/4 of the strains. It was also synergistic for 40% of the *P. aeruginosa* strains and partially synergistic for half of them; moxalactam MIC's including those of 128 $\mu\text{g/ml}$ decreased to 16 $\mu\text{g/ml}$ or less. Amikacin-piperacillin was synergistic for more than 90% of the enterobacteria and 1% was indifferent to the combination. The MIC's of piperacillin resistant strains were reduced to 50 $\mu\text{g/ml}$ or less. This combination was also synergistic for about 70% of *P. aeruginosa* strains and partially synergistic for 28%; all piperacillin MIC's decreased to 3.13 $\mu\text{g/ml}$ or less. Most enterobacteria were indifferent to the moxalactam-piperacillin combination because of the previously described moxalactam problem. However, among *Serratia* strains (50%), there was no change in the MIC's to either agent; all piperacillin-resistant strains were in this group. The combination exerted a synergistic effect on 6% of the enterobacteria and was partially synergistic for the remaining strains. It was also synergistic or partially so for 30% and 50% of *P. aeruginosa* strains. Moxalactam MIC's became 64 $\mu\text{g/ml}$ or less and most piperacillin MIC's decreased to 6.25 $\mu\text{g/ml}$ or less.

Various empiric antibiotic regimens are used to treat infections in this patient population. As these antibiotics are usually used in combination, the in vitro effectiveness of these combinations against bacteria recovered while a particular regimen is in progress is determined by a checkerboard microdilution method on a routine basis. The current synergy panels contain ticarcillin-amikacin and ticarcillin-moxalactam.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06275 11 COB
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PERIOD COVERED
July 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cell Transfusion Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any)

Community Blood and Plasma Service, Baltimore, Maryland
Dr. Paul Tersaki, Univ. of California, Los Angeles;
The University of Maryland, Department of Nuclear Med.; Baltimore PH Typing Lab

LAB/BRANCH

Clinical Oncology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH Baltimore, MD 21201

TOTAL MANYEARS:

9

PROFESSIONAL:

4.5

OTHER:

4.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS

☐ (b) HUMAN TISSUES

☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Management of alloimmunized patients has been facilitated by further expansion of the HLA matched donor pool and continued growth of the program of autologous frozen platelet transfusion. Studies of patients with non-lymphocytic leukemia have demonstrated that the development of alloimmunization is not related to the number of platelet transfusions received. With few exceptions, patients who did not develop lymphocytotoxic antibody within 6-8 weeks of platelet transfusion never developed antibody despite subsequent transfusions, possibly suggesting a state of immune tolerance to histocompatibility antigens. A randomized study comparing the rate of alloimmunization in patients receiving lymphocyte depleted and "standard" platelet concentrations is in progress. Platelet cryopreservation using glycerol-glucose as the cryoprotective agents has demonstrated good freeze-thaw recovery but significant loss of ATP and morphologic integrity. Indium labeled granulocytes migrate to infected sites within 30 min. of transfusion and have been shown to be a relatively simple and reliable label permitting excellent scanning and kinetic analysis. Studies are in progress using this technique to determine the effect of alloimmunization on granulocyte migration post transfusion.

The Cell Component Therapy Section provides platelet and granulocyte transfusion for the treatment and prophylaxis of infections and hemorrhagic applications in patients with leukemia and other type of cancer receiving aggressive therapies. In addition, an active laboratory interested in various aspects of platelet and granulocyte function is in operation.

1. Platelet transfusion

Approximately 14,000 units of platelet concentrate were transfused in 1978/79. Approximately 4500 units were collected and processed by the Cell Component Therapy Section and consisted of HLA-matched platelets, platelets administered with granulocyte transfusion and frozen platelets. The remainder of the platelets transfused were either HLA-matched platelets or random-donor platelets obtained by two unit plateletpheresis performed by the Community Blood & Plasma Service. Alloimmunization to platelet remains the greatest clinical problem in the field of platelet transfusion and the transfusions of HLA-matched platelets was greatly expedited by the recruitment by Community Blood & Plasma Service of approximately 1,000 HLA-matched donors which supplements a file of approximately 700 active donors with the BCRP. HLA-matched donors for granulocyte transfusion are also available from this source.

There is a major ongoing interest in studies of the prevention or delay of onset of alloimmunization. An analysis was undertaken to determine whether a dose response relationship exists between the number of platelets administered and the frequency of alloimmunization during the induction therapy of acute nonlymphocytic leukemia. A group of 106 patients was analyzed and the overall rate of alloimmunization was 38%. It should be noted that patients who were alloimmunized on admission, who received granulocytes or who had a rapid development of antibodies suggesting an anamnestic response were not included in this study. No dose response relationship was evident and the incidence and titre of lymphocytotoxic antibody was similar in patients receiving either four or more than four platelet transfusions. There is no apparent clinical difference between the patients who made and did not make antibody and in particular, there was no specific time relationship between the initiation of chemotherapy and the first platelet antigenic exposure. The implication of this study is that platelets should not be withheld expressly for the purpose of preventing alloimmunization because alloimmunization appears to be related to individual host factors which cannot be identified at present. Long-term analysis of 67 patients who did not make antibody during the first eight weeks of therapy was also undertaken. Of note is that only six patients subsequently make antibody despite multiple exposure to platelets, red blood cells and granulocytes, suggesting that a form of immune tolerance to histocompatibility antigens had developed. Again, the clinical implication is that prophylactic platelets should not

be withheld from patients during maintenance and reinduction because patients seem to make antibody initially or not at all. A wide variety of clinical factors were analyzed in this group of patients and there is no apparent difference which could be discerned from the larger group of patients who in fact became alloimmunized.

Platelet cryopreservation remains one of the major interests of the unit and last year approximately 1200 units of autologous frozen platelets were administered to a total of 45 patients. Of note is that 21 of these patients were alloimmunized, and 9 had no other donors available. The results of frozen platelets using 5% DMSO as a cryoprotective agent have remained remarkably similar throughout the years with corrected count increments averaging 13,700 which represent a recovery of approximately two-thirds of that expected of normal platelets. A large number of variables which may affect these results have been studied in our laboratory. There was no relationship between the donors' pre-freezing modal platelet volume or platelet count, the modal post thawing volume and the subsequent platelet recovery. There was a good correlation between estimates of platelet morphology and in vivo recovery. Because of the possibility that platelets of different age and size might have different tolerance to freezing injury, a method of separating platelets of different size on a density gradient is being done with the eventual goal of freezing these platelets separately. Using this technology it should also be possible to do in vitro platelet function studies of different populations of platelets in patients with myeloproliferative disorders in whom large numbers of large, morphologically abnormal platelets tend to circulate. Lastly, experiments are in progress using a new glycerol-glucose method of platelet freezing to see if the DMSO results can be improved upon. Preliminary results indicate that very few platelets are lost during the freeze-thaw procedure. ATP levels are only about 50% of pre-freeze controls, however, and morphologic evaluation demonstrates significant deterioration after freezing which preliminarily appears worse than the DMSO product.

In the last year an IBM 297 Blood Processor was purchased. Although this machine has been used predominantly for granulocyte transfusion it is an effective machine for platelet collection and studies of cryopreservation of HLA-matched and autologous frozen platelets using this machine are in progress.

A provocative study from Holland has suggested that platelet concentrates rendered lymphocyte poor by centrifugation are less immunogenic than standard platelet concentrates. This was not a prospectively randomized trial and an attempt is being made to confirm these results during initial induction therapy of the patients with nonlymphocytic leukemia. Eligible patients are

randomized to receive either standard platelet concentrate administered for the usual clinical indications compared to platelets which have been rendered lymphocyte poor by subsequent centrifugation. Approximately 10^7 (90 % depletion) lymphocytes are included in this latter preparation which also produces about a 20% platelet loss. The implications in terms of the importance of reducing alloimmunization are obvious and to date approximately 35 patients have been randomized on this study.

Because lymphocytotoxic and other antiplatelet antibody tests are not usually available on short notice in most hospitals a simple clinical assessment of the diagnosis of alloimmunization would be helpful. An analysis of the causes of failure to achieve adequate increment 24 hours after transfusion was done and compared with the results of one hour post transfusion counts in the same group of patients. It was noted that with the exception of massive splenomegaly or gram negative shock, alloimmunization was the only cause of such rapid platelet destruction that corrected count increment of less than 10,000 occurred at one hour. Twenty-four hour counts were valueless, however, in distinguishing between alloimmunization and other causes of rapid platelet destruction such as fever, infection and DIC. This impression was confirmed by the fact that HLA-matched transfusions were effective only in patients in whom one hour post transfusion counts were low with results comparable to random donor platelets in patients in whom one hour post transfusion counts were satisfactory. Thus, a count one hour post transfusion of random donor platelets provides a good and simple clinical guideline suggesting the requirement for HLA-matched platelets. A number of tests have been developed recently allowing for the measurement of platelet associated antibody as a means of detecting antiplatelet antibody. None have been prospectively used for cross-matching for selection of donors however. An ELISA assay using alkaline phosphatase linked to antihuman immunoglobulin is being set up in the laboratory for possible use in donor selection. The test is relatively simple and has the advantage of using fixed platelets which can be stored for days to weeks prior to testing.

2. Granulocyte transfusion therapy

Therapeutic granulocyte transfusions are administered as early in the course of serious infection as possible, particularly in patients with gram negative bacteremia or infections in parenchymal organs. Transfusions are administered only to severely (less than $100/\text{mm}^3$) granulocytopenic patients in whom long periods of granulocyte aplasia are anticipated. Granulocytes are collected using either the Haemonetics Model 30 or the the IBM 2997. All donors receive hydroxyethyl starch and corticosteroids as premedication in order to maximize granulocyte dose. It has been noted that the yield of granulocytes obtained from the IBM

2997 (24×10^9) is approximately twice that obtained from the Haemonetics and every attempt is made to use this machine therefore for granulocyte collection. In addition, observations have been made on four patients receiving large doses of granulocytes from patients with chronic myelogenous leukemia. Sustained increases in granulocyte count have been noted in three nonalloimmunized recipients of these transfusions with documentation of short term engraftment by Philadelphia chromosome studies. Of note was that some of these patients maintained granulocytes counts for two weeks which provided both treatment of the original infection as well as apparent prophylaxis against subsequent infection. No graft versus host disease was observed, and whenever appropriate CML donors are available they are used for severely infected patients.

A study of prophylactic granulocyte transfusion was completed and recently published. The study was stopped early because of an excessive (70%) rate of transfusion reactions and alloimmunization in the group receiving granulocytes. Our rate of alloimmunization has been confirmed in subsequent studies at other institutions. It is our feeling that prophylactic granulocytes should be utilized only in strictly investigational settings because the risk of alloimmunization with difficulties in subsequent platelet and white cell support outweigh any marginal benefit in terms of infection prevention. Although there was no statistically significant difference between the transfusion and control groups, there was a higher incidence of aspergillus infections in the group receiving platelets alone. No difference in survival or complete remission was noted.

In addition to problems with an inadequate granulocyte dose, questions related to the importance of histocompatibility testing for granulocyte transfusion remain. It is presently our practice not to administer granulocyte transfusions to patients who are known to be alloimmunized unless suitably matched donors are available. Present granulocyte antibodies screening tests are cumbersome and assessment of a new semi-quantitative method measuring adherence of Cowans strain staphylococcus (which binds to IgG) to neutrophils fixed to glass slides is in progress. An exchange of sera with Dr. Lalezai from New York to test for possible granulocyte specific activity of the sera from the prophylactic granulocyte transfusion study is also contemplated.

In an effort to quantitatively measure the effect of alloimmunization on post transfusion granulocyte behavior, ¹¹¹Indium-labeled granulocytes are being studied. Approximately 60% labeling of granulocytes using a simple separation and labeling procedure with preservation of normal granulocyte morphology, viability and function has been developed. Studies of 10 non-alloimmunized patients has revealed prompt localization of

granulocytes in infected areas, some within a half-hour of infusion. This observation provides some insight into the well known phenomenon of much decreased recovery of granulocytes post transfusion to infected patients. It also confirms that the labeling and transfusion procedure, which involves the transfusion of only 10^8 granulocytes which is obtainable from 50 ml of peripheral blood, does provide an effective marker. In studies of 2 non-alloimmunized patients using mismatched granulocytes, no migration to infected sites was seen, thereby preliminarily confirming the long-held clinical impression about the importance of matching for granulocyte transfusion for alloimmunized patients. Further studies are obviously needed and in progress and will also include comparison of different leukocyte cross-matching tests to determine which are most predictive of subsequent transfusion behavior. Further implications of this technology include the use of autologous labeled granulocyte for abscess localization.

3. Other Laboratory Functions

- A. Lymphocyte and blast collection for other DCT laboratories in Baltimore and Bethesda.
- B. Cryopreservation of blasts from patients with leukemia for immunotherapy studies. Blasts are cryopreserved using 10% DMSO with recoveries postthaw of approximately 70% which are quite adequate for neuraminidase treatment for use in the BCRP ANLL Protocol.
- C. Therapeutic plasmapheresis of a few patients have been performed including two patients with myasthenia gravis, one patient with Guillain-Barre Syndrome and another with systemic lupus erythematosus in association with immune thrombocytopenia and renal failure. It is expected that more plasmapheresis may be done in the future as the IBM 2997 is an extremely efficient machine requiring only about 90 minutes to perform a whole body plasma exchange.
- D. Large scale collection of immune plasma from donors recovering from chicken pox or herpes zoster continues to be done.

Additional Investigations:

- A. In vitro test of bleeding time: an apparatus developed and supplied by Payton Instrument Company was studied which had the putative capacity to perform repeated in vitro assessments of bleeding time. This would have obviated the need to perform multiple template bleeding times on granulocytopenic patients. Unfortunately the results of the machine were not at all reproducible despite many alterations in technique and blood anticoagulant. In addition, the machine as designed is extremely difficult to

set up and clean and posed a substantial hepatitis risk because of the amount of blood that was splattered. Further tests of this machine are no longer being done.

- B. An analysis of the results of platelets which have been stored for variable lengths of time at room temperature is in progress. There is surprisingly little literature on the subject except for the few original studies documenting the feasibility of storing platelets for 72 hours at ambient temperature.
- C. An analysis of the results of a large number of HLA-matched transfusions is in progress with a particular emphasis on the measurement of the level of lymphocytotoxic antibody and the results of partially HLA-mismatched transfusions. The working hypothesis is that transfusions selectively mismatched for cross reactive antigens are most likely to be successful in patients with lower levels of antibody but that perfectly matched platelets are necessary for patients with high antibody titers.
- D. In 4 of 4 patients in whom engraftment occurred following transfusion of leucocytes from patients with CML, it was documented that levels of leukocyte alkaline phosphatase (LAP) rose in the circulating granulocytes. This suggests that the low LAP seen in CML is not an intrinsic property of the abnormal clone but in fact there seems to be an important "environmental" influence on the formation of the enzyme.
- E. Stem cells from the circulating blood of selected patients with CML are being frozen using 10% DMSO, after ficoll-hypaque separation for possible subsequent use as autologous reconstitution should blast crisis develop.

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PROJECT DESCRIPTION

Z01 CM 06275 11 COB

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06043-09 COB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Automated Monitoring of Drug Therapy		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Clarence L. Fortner, M.S. OTHER: William R. Grove, B.S. Larry Yamamoto, M.D. Peter H. Wiernik, M.D.	Head, Clinical Research Pharmacy Section Assistant Director, Clinical Research Pharmacy Section Acting Chief, Health Services Research, USPHS Hospital Chief, Clinical Oncology Branch	COB-NCI COB-NCI HSR-NIH COB-NCI
COOPERATING UNITS (if any) Health Services Research, USPHS Hospital, Baltimore, MD		
LAB/BRANCH Clinical Oncology Branch		
SECTION Clinical Research Pharmacy Section		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 1.25	PROFESSIONAL: .25	OTHER: 1.0
CHECK APPROPRIATE BOX(ES)		
☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
The objective of this project is to provide an efficient and rapid method of <u>automated</u> collecting of data concerning the clinical trials of experimental drugs. The system of <u>computer</u> processing of <u>drug administration</u> information provides accurate periodic <u>summaries</u> of all BCRP patients' <u>drug therapy</u>. Each drug card is typed containing the name of the drug, dose, route of administration, frequency, date, and patient's name and medical record number. Each card also contains the direct <u>administration</u> record as charted by the nurses at the time of administration. These cards are processed by optical character recognition and a weekly summary of all drugs ordered and their administration record, organized by patient and by problem, are produced. This system fulfills all the requirements of drug ordering, administration and recording in addition to providing machine-readable data for computer input. This system eliminates the transcription operations usually associated with preparation of input for computers and provides a data pool for clinical studies.		

The objective of this project is to provide an efficient and rapid method of collecting data concerning the clinical trials of experimental drugs. This is being studied by a computerized method of ordering and recording all drug therapy.

A system of processing drug administration information provides accurate, weekly summaries of each patient's complete drug therapy. The physician writes each drug order directly on a medication card, producing three copies: the pharmacy copy (for entry to a patient's drug profile), a copy for medical records and a copy for automated analysis. The nurse charts medications given directly on the first copy of the drug form at the time of drug administration. This data is read weekly by optical character recognition. The data is collated and patient drug summaries are printed listing drug, dose, total dose, frequency of administration and a daily record of administration or omission of each drug. A monthly drug utilization report is also obtained as a by-product.

The problem number and statement according to the Weed's problem oriented health record system has been incorporated into the form which allows each drug to be listed by problem number on the weekly drug summary for each patient. Space on the drug form has also been allotted for the patient's height, weight, and body surface area. This gives the pharmacist enough data to recalculate all drug doses listed in research protocols. Not only does this recalculation protect the patient, but it also protects the research environment by insuring the accuracy of all doses administered. This system fulfills all the requirements of drug ordering, administration, and recording, in addition to providing machine-readable data for computer input. The summaries are checked by the pharmacists, and become a permanent record of drug therapy both in the patient's chart and in the pharmacy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration. A complete list of all drugs administered to the patient are recorded by problem numbers in a concise and orderly fashion in the patient chart. This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06237-09 COB									
PERIOD COVERED October 1, 1979 to September 30, 1980											
TITLE OF PROJECT (80 characters or less) Development of a Clinical Research Pharmacy Section											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT											
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Clarence L. Fortner, M.S.</td> <td style="width: 33%;">Head, Clinical Research Pharmacy Section</td> <td style="width: 33%; text-align: right;">COB NCI</td> </tr> <tr> <td>OTHER: William R. Grove, B.S.</td> <td>Assistant Director, Clinical Research Pharmacy Section</td> <td style="text-align: right;">COB NCI</td> </tr> <tr> <td>Rebecca Finley, Pharm.D.</td> <td>Clinical Pharmacist</td> <td style="text-align: right;">COB NCI</td> </tr> </table>			PI: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB NCI	OTHER: William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB NCI	Rebecca Finley, Pharm.D.	Clinical Pharmacist	COB NCI
PI: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB NCI									
OTHER: William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB NCI									
Rebecca Finley, Pharm.D.	Clinical Pharmacist	COB NCI									
COOPERATING UNITS (if any) None											
LAB/BRANCH Clinical Oncology Branch											
SECTION Clinical Research Pharmacy Section											
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201											
TOTAL MANYEARS: 3	PROFESSIONAL: 3	OTHER: 0									
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SUMMARY OF WORK (200 words or less - underline keywords)											
The objective of this study is to determine the role of the pharmacist in the <u>patient care/research areas</u>, and as a member of the health care team actively contributing to the patient's care and research efforts. The pharmacist, utilizing new methods, has become a valuable member of the research health care team contributing directly to patient care and research. The following has been accomplished: improved chemotherapy records, monitored chemotherapy orders against protocols, improved drug prescribing practices by the promotion of rational and safe drug use, the prevention of potential drug-drug interactions, the detection and prevention of adverse drug reactions, the detection of drug-induced laboratory test abnormalities, the detection of possible drug-induced disease, the provision for easily accessible drug information, the continuation of education for various members of the health care team, counseling of the patient on proper use of drugs, and the detection and prevention of intravenous admixture incompatibilities.											

The objective of this study is to determine the role of the pharmacist in a research-patient care area.

The pharmacist is located in the midst of the patient care area in order to allow him to investigate new concepts, approaches, and research opportunities to aid in the care of the patient. The pharmacist attends morning and evening ward rounds and infectious disease rounds, reviews charts, interviews patients and is familiar with the patients' condition. Pharmacy rounds are convened immediately after the morning medical rounds. All pharmacists attend, as all new problems are reviewed. This provides the pharmacists with all of the patient current problems; therefore, he can intelligently discuss therapy with the patient's physicians during the working day, as the need occurs. With this information, he can also provide the staff with unsolicited information.

An integral part of this service is the therapy surveillance system. The nucleus of the drug surveillance system is the individual patient drug profile and investigational drug record. The pharmacist receives a direct copy of the physician's drug order and transcribes it to the patient's drug profile and chemotherapy record. The drug record includes the patient's name, primary and secondary problems, physician's name, known allergies or idiosyncrasies and all drug orders. The pharmacist reviews each new drug order for completeness and recalculates all protocol drug dosages and then reviews the drug profile against the new order for drug interactions, laboratory problems, dietary problems, and possible adverse drug reactions. The drugs are then dispensed through a unit dose system which provides accurate records. All returned doses to the pharmacy are investigated for reason of non-compliance. The monitoring system includes evaluating the necessity of continued medication orders, pharmacokinetics, proper drugs, and correct dosage.

The pharmacist, utilizing the above methods, has become a valuable member of the research health care team and contributes directly to the care of the patient. The following has been accomplished: improved chemotherapy records, monitored chemotherapy orders against protocols, improved drug prescribing practices by promoting the safe and rational use of drugs, the prevention of potential drug-drug interactions, the detection and prevention of adverse drug reactions, the detection of drug-induced laboratory test abnormalities, the detection of possible drug-induced diseases, the provision of easily accessible drug information, counseling of the patient on proper use of drugs, and the detection and prevention of intravenous admixture incompatibilities. This has reduced drug exposure to the patient, decreased nursing and pharmacy time spent administering and preparing drugs, and reduced cost.

A higher quality of drug prescribing and reduced medication errors has contributed toward better research/patient care and has enhanced the quality of the research data.

Drug information is becoming a major by-product of locating the pharmacist in the patient care area. This information is being requested by all members of the health care team. Continuing education is also involved in this process of providing drug information. The impact of this service will continue to be studied by the audit process.

The pharmacist is making rounds with a nurse to improve compliance of patients taking non-absorbable oral antibiotics and other medications. A pharmacist is doing audiograms of patients receiving potentially ototoxic agents.

Present studies will be continue, and additional methods to improve patient care will be further researched.

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6. Fortner, C.L. and Grove, W.R., Complications and Toxic Manifestations of Cancer Therapy. Oncology Pharmacy Practice: Proceedings of the American Society of Hospital Pharmacists Institute. New York, Elsevier-North Holland, Inc., pp. 9-23, 1980.
7. Grove, W.R. and Fortner, C.L., Current Concepts in Medical Management of Pain. Oncology Pharmacy Practice: Proceedings of the American Society of Hospital Pharmacists Institute. New York, Elsevier-North Holland, Inc., pp. 51-69, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06912-06 COB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Antineoplastic Drug Displays for the Computerized Problem-Oriented Medical Information System		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: William R. Grove, B.S. Clarence L. Fortner, M.S. OTHER: Judy Gundel, B.S. Robert Esterhay, M.D. Peter H. Wiernik, M.D.	Assistant Director, Clinical Research Pharmacy Section Head, Clinical Research Pharmacy Section Clinical Pharmacist Senior Investigator, Medical Oncology Section Chief, Clinical Oncology Branch	COB-NCI COB-NCI COB-NCI COB-NCI COB-NCI
COOPERATING UNITS (if any) PROMIS Laboratory, Dept. of Medicine, University of Vermont		
LAB/BRANCH Clinical Oncology Branch		
SECTION Clinical Research Pharmacy Section		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: .6	PROFESSIONAL: .6	OTHER: 0
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this project is to prepare referenced <u>summarized pharmacologic</u> information which is readily retrievable at the touch-sensitive <u>cathode ray screen terminal</u> located at the BCRP nursing station. Current literature is reviewed to obtain accurate and complete drug information on <u>antineoplastic agents</u> . The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Section of the BCRP or the PROMIS Laboratory, University of Vermont. After completion, <u>display sequences</u> are sent to the PROMIS laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the <u>computerized system</u> . The antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.		

The objectives of this project are to prepare referenced summarized pharmacologic information which is readily retrievable at the touch-sensitive cathode ray screen terminal located at the BCRP nursing station. These displays are to be complete, concise, and accurate summaries of currently-available literature. Additional antineoplastic drugs are added to the computerized drug displays already on file with the PROMIS system, Burlington, Vermont.

Current literature is reviewed to obtain accurate and complete drug information on antineoplastic agents. This information is then organized to a specific format to conform with the over 20,000 displays currently included in the PROMIS system. The standardized format consists of separate display pages for information as follows: DRUG NAME, CHECK PROBLEM LIST FOR, SIDE EFFECTS TO WATCH FOR, DRUG INTERACTIONS, TEST INTERFERENCES, USUAL DOSAGE, PHARMACEUTICAL DATA, MECHANISM OF ACTION, AND METABOLISM AND EXCRETION. Reference codes are included with all pages of information, which may be further explored by contacting either the Clinical Research Pharmacy Section of the BCRP or the PROMIS Laboratory, University of Vermont. After completion, display sequences are sent to the PROMIS Laboratory to be audited for accuracy, completeness, and correctness of clinical judgement before being entered into the computerized system. Previously entered data may be updated as necessary by providing the new information and references to the PROMIS laboratory for incorporation.

The completed antineoplastic drug displays provide ready access to referenced summaries of the current medical literature. They form an integral part of the contribution to improved patient care and improved medical records played by the PROMIS system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06943-05 COB						
PERIOD COVERED October 1, 1979 to September 30, 1980								
TITLE OF PROJECT (80 characters or less) Audit of the Outpatient Drug Profile								
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: William R. Grove, B.S.</td> <td style="width: 40%;">Assistant Director, Clinical Research Pharmacy Section</td> <td style="width: 20%; text-align: right;">COB-NCI</td> </tr> <tr> <td>OTHER: Clarence L. Fortner, M.S.</td> <td>Head, Clinical Research Pharmacy Section</td> <td style="text-align: right;">COB-NCI</td> </tr> </table>			PI: William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB-NCI	OTHER: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB-NCI
PI: William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB-NCI						
OTHER: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB-NCI						
COOPERATING UNITS (if any) None								
LAB/BRANCH Clinical Oncology Branch								
SECTION Clinical Research Pharmacy Section								
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201								
TOTAL MANYEARS: .6	PROFESSIONAL: .6	OTHER: 0						
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) HUMAN SUBJECTS</td> <td>☐ (b) HUMAN TISSUES</td> <td>☒ (c) NEITHER</td> </tr> <tr> <td colspan="3">☐ (a1) MINORS ☐ (a2) INTERVIEWS</td> </tr> </table>			☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER	☐ (a1) MINORS ☐ (a2) INTERVIEWS		
☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER						
☐ (a1) MINORS ☐ (a2) INTERVIEWS								
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to promote compliance and knowledge of <u>state pharmacy laws</u> through an <u>audit of the outpatient prescription file</u>. The process audited is the pharmacist's completion of the required information on the prescription. The prescriptions from one day's outpatient prescription file were audited for complete drug information (drug name, dose, quantity, directions), patient name, physician signature, date, complete filing information (manufacturer, lot number, expiration date, pharmacist's initials). In addition, a lot number verification of the source was conducted. For controlled substance prescriptions, the audit also included the patient's address, physician, DEA/SS number, sequential prescription numbers, and the red "C". Forty-five prescriptions were audited. The following results were obtained: Drug name, 45/45; Dose, 43/45; Quantity, 41/45; Directions, 42/45; Date, 41/45; Manufacturer, 30/45; Lot number, 43/45 and Expiration date, 38/45. Items which were not being completely filled out were brought to the attention of the staff. Additional audits will be performed to monitor compliance.								

The purpose of this study is to promote compliance and knowledge of state pharmacy laws through an audit of the outpatient prescription file. The process audited is the pharmacist's completion of the required information on the prescription.

The prescriptions from one day's outpatient prescription file were audited for complete drug information (drug name, dose, quantity, directions), patient name, physician signature, date, complete filing information (manufacturer, lot number, expiration date, pharmacist's initials). In addition, a lot number verification of the source was conducted. For controlled substance prescriptions, the audit also included the patient's address, physician, DEA/SS number, sequential prescription numbers, and the red "C".

Forty-five prescriptions were audited. The following results were obtained: Drug name, 45/45; Dose, 43/45; Quantity, 41/45; Directions, 42/45; Date, 41/45; Manufacturer, 30/45; Lot number, 43/45 and Expiration date, 38/45. Items which were not being completely filled out were brought to the attention of the staff. Additional audits will be performed to monitor compliance.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06961-04 COB															
PERIOD COVERED October 1, 1979 to September 30, 1980																	
TITLE OF PROJECT (80 characters or less) Establishment of a Pharmacy Clinical Clerkship in Oncology																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																	
<table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">PI: Clarence L. Fortner, M.S.</td> <td style="width: 45%;">Head, Clinical Research Pharmacy Section</td> <td style="width: 20%; text-align: right;">COB-NCI</td> </tr> <tr> <td>OTHER: William R. Grove, B.S.</td> <td>Assistant Director, Clinical Research Pharmacy Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Robert Kerr, Pharm.D.</td> <td>Director, Clinical Pharmacy, University of Maryland</td> <td></td> </tr> <tr> <td>Rebecca Finley, Pharm.D.</td> <td>Clinical Pharmacist</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td style="text-align: right;">COB-NCI</td> </tr> </table>			PI: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB-NCI	OTHER: William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB-NCI	Robert Kerr, Pharm.D.	Director, Clinical Pharmacy, University of Maryland		Rebecca Finley, Pharm.D.	Clinical Pharmacist	COB-NCI	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI
PI: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB-NCI															
OTHER: William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB-NCI															
Robert Kerr, Pharm.D.	Director, Clinical Pharmacy, University of Maryland																
Rebecca Finley, Pharm.D.	Clinical Pharmacist	COB-NCI															
Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI															
COOPERATING UNITS (if any) University of Maryland, School of Pharmacy																	
LAB/BRANCH Clinical Oncology Branch																	
SECTION Clinical Research Pharmacy Section																	
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201																	
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0															
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) The Clinical Research Pharmacy Section of the BCRP has joined with the University of Maryland School of Pharmacy in establishing a <u>clinical clerkship</u> in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective four week rotation. The objective is to provide a clinical experience in <u>oncology</u>. The students are to understand the criteria for oncology research/treatment protocols; i.e., patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities, and the management of non-neoplastic concurrent medical/social problems. The pharmacy student should gain experience in both the inpatient and ambulatory areas.																	

The Clinical Research Pharmacy Service has joined with the University of Maryland School of Pharmacy in establishing a clinical clerkship in oncology for students obtaining their Doctor of Pharmacy Degree. This clerkship is an elective four week rotation.

The objectives are to provide a clinical experience in oncology. The students are to understand the criteria for oncology research/treatment protocols; i.e., patient selection, study design and evaluation of study. They will gain experience in cancer therapy, toxicities and the management of non-neoplastic concurrent medical/social problems. They will learn the effective and reliable reference sources for the most current information on cancer chemotherapy.

These students will be directed through the Clinical Research Pharmacy Service with support from the Clinical Oncology Branch.

This program had six rotations during the past year and will continue for further evaluation.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06990-03 COB												
PERIOD COVERED October 1, 1979 to September 30, 1980														
TITLE OF PROJECT (80 characters or less) The Role of the Pharmacist in Monitoring Patients Receiving Ototoxic Drugs														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: Freda Danhauer, B.S.</td> <td style="width: 40%;">Clinical Pharmacist</td> <td style="width: 30%; text-align: right;">COB-NCI</td> </tr> <tr> <td>OTHER: Clarence L. Fortner, M.S.</td> <td>Head, Clinical Research Pharmacy</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Stephen C. Schimpff, M.D.</td> <td>Head, Infection Research Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td style="text-align: right;">COB-NCI</td> </tr> </table>			PI: Freda Danhauer, B.S.	Clinical Pharmacist	COB-NCI	OTHER: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy	COB-NCI	Stephen C. Schimpff, M.D.	Head, Infection Research Section	COB-NCI	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI
PI: Freda Danhauer, B.S.	Clinical Pharmacist	COB-NCI												
OTHER: Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy	COB-NCI												
Stephen C. Schimpff, M.D.	Head, Infection Research Section	COB-NCI												
Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI												
COOPERATING UNITS (if any) None														
LAB/BRANCH Clinical Oncology Branch														
SECTION Clinical Research Pharmacy Section														
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201														
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0												
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this study is to evaluate the feasibility of pharmacist performed <u>audiometric</u> monitoring of patients who are receiving potentially <u>ototoxic</u> therapy. Patients receiving potentially ototoxic drugs have baseline pure tone audiograms performed by the pharmacist utilizing a portable audiometer. A follow-up audiogram is obtained at the completion of therapy. All abnormal follow-up audiograms are noted and brought to the attention of the physician. Additional follow-up audiograms are scheduled in those patients with abnormal results to determine the reversibility of the toxicity. This function will allow the pharmacist significant patient contact and a greatly increased awareness of the need for a multidisciplinary approach to patient care. This function will also provided a significant opportunity for the patient to utilize the pharmacist as a source person concerning their drug therapy. This study will investigate how the pharmacist can contribute significantly to the care of the patient by performing clinically relevant audiometric studies of patients at high risk of developing ototoxicity.														

This study was designed to evaluate the feasibility of having a pharmacist conduct pure-tone audiometric studies on patients receiving potentially ototoxic drugs. All audiograms are conducted in the patient's semi-private room utilizing a portable beltone audiometer (Model 10D).

Training of a pharmacist was accomplished in conjunction with the audiology department of the University of Maryland Hospital. This pharmacist, in turn, educated the other pharmacists in the proper technique of obtaining a pure-tone audiogram.

The pharmacist is responsible for obtaining baseline and follow-up audiograms on all patients randomly allocated to receive empiric antibiotic therapy which includes an aminoglycoside or other potential ototoxic drugs; i.e., cisplatin. Abnormal findings are related to the patients' physicians.

The pharmacist has been able to demonstrate a 5% overall occurrence of ototoxicity associated with aminoglycoside antibiotics.

This function has given the pharmacist a significant and productive amount of patient contact time and interaction with research protocols. Not only has this increased the pharmacist's awareness of the need for a multidisciplinary approach to patient care and research data collection, but it has increased the patient's utilization of the pharmacist as a drug information source person.

This study will investigate the ability of the pharmacist to contribute to the care of the patient and to the compilation of research data by conducting accurate audiometric studies of patients receiving ototoxic agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06996-02 COB												
PERIOD COVERED October 1, 1979 to September 30, 1980														
TITLE OF PROJECT (80 characters or less) Meperidine Hydrochloride for the Treatment of Shaking Chills & Fever														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT														
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: Rebecca Finley, Pharm.D.</td> <td style="width: 40%;">Clinical Pharmacist, Clinical Research Pharmacy Section</td> <td style="width: 30%; text-align: right;">COB NCI</td> </tr> <tr> <td>OTHER: Joseph Aisner, M.D.</td> <td>Head, Medical Oncology Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Clarence L. Fortner, M.S.</td> <td>Head, Clinical Research Pharmacy Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td style="text-align: right;">COB-NCI</td> </tr> </table>			PI: Rebecca Finley, Pharm.D.	Clinical Pharmacist, Clinical Research Pharmacy Section	COB NCI	OTHER: Joseph Aisner, M.D.	Head, Medical Oncology Section	COB-NCI	Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB-NCI	Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI
PI: Rebecca Finley, Pharm.D.	Clinical Pharmacist, Clinical Research Pharmacy Section	COB NCI												
OTHER: Joseph Aisner, M.D.	Head, Medical Oncology Section	COB-NCI												
Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB-NCI												
Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI												
COOPERATING UNITS (if any) None														
LAB/BRANCH Clinical Oncology Branch														
SECTION Clinical Research Pharmacy Section														
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201														
TOTAL MANYEARS: .2	PROFESSIONAL: .2	OTHER:												
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER														
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this study is to 1) determine if <u>meperidine hydrochloride</u> is useful in the treatment of <u>shaking chills</u> and <u>fever</u> following <u>amphotericin B</u> infusions, 2) to determine if using meperidine is more effective than simply discontinuing the amphotericin B, and 3) to determine the complications which might arise from the use of meperidine given for this purpose. Seven patients have been randomized on multiple occasions for a total of 19 reactions. In the meperidine group, 9/9 reactions stopped within 30 minutes of the administration of meperidine, with a mean cessation time of 10.8 minutes. The placebo group had a mean time of 37.4 minutes to cessation of reaction with 3 out of 10 subsiding spontaneously.														

Meperidine hydrochloride is being evaluated in a prospective double-blind study for its effectiveness in stopping shaking chills occurring with amphotericin B infusions. Amphotericin B infusions were prepared with 25 mg of hydrocortisone sodium succinate and 500 units of sodium heparin added to 500 ml of 5% dextrose in water. If a severe reaction occurred, the amphotericin B infusion was stopped and an infusion of 5% dextrose in water was slowly administered for 5-10 minutes. If the reaction did not spontaneously subside, 1/2 ml of the coded preparation was slowly administered, either 50 mg of meperidine or an equal volume of isotonic saline. The amount of time necessary to stop the reaction was recorded. If after an additional 10-15 minutes the shaking chills and fever had not subsided, more of the coded preparation was given slowly. The time to reaction, the amount of drug or placebo administered, and the time of cessation of shaking chills were recorded. Seven patients have been randomized on multiple occasions for a total of 19 reactions. In the meperidine group, 9/9 reactions stopped within 30 minutes of the administration of meperidine with a mean cessation of 10.8 minutes. The placebo group had a mean time of 37.4 minutes to cessation of reaction with 3 of the 10 reactions subsiding spontaneously. The comparison between meperidine and placebo for cessation of reaction within 30 minutes and the mean time of cessation of reaction was significantly different, with a p value of less than .0025 and a p value of less than .025, respectively. Side effects with meperidine have been minimal and less severe than the shaking chills and fever seen with the amphotericin B infusions. Administering meperidine hydrochloride appears to eliminate these reactions more effectively and more rapidly than simply discontinuing the amphotericin B. The present study is complete.

PUBLICATIONS:

Burks, L., Aisner, J., Fortner, C. and Wiernik, P. Meperidine Hydrochloride for the Treatment of Shaking Chills and Fever, Arch. Intern. Med. 140:483-484, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06997-02 COB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Comparative Auditory Toxicity of Aminoglycoside Antibiotics in Leukopenic Patients		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Clarence L. Fortner, M.S. OTHER: Freda Danhauer, B.S. Stephen C. Schimpff, M.D. William R. Grove, B.S. Peter H. Wiernik, M.D.	Head, Clinical Research Pharmacy Clinical Pharmacist Head, Infection Research Section Assistant Director, Clinical Research Pharmacy Section Chief, Clinical Oncology Branch	COB-NCI COB-NCI COB-NCI COB-NCI COB-NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Oncology Branch		
SECTION Clinical Research Pharmacy Section		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: .2	PROFESSIONAL: .2	OTHER:
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this study is to assess the relative incidence of auditory toxicity among three different <u>aminoglycosides</u>. <u>Audiograms</u> are to be obtained by a pharmacist utilizing a portable <u>audiometer</u>. It is our intention to study the monitoring function as performed by a pharmacist as well as to present the results of the auditory toxicity portion of the study. Thirty-two patients received ticarcillin (T) and gentamicin (G), 29 received ticarcillin and netilmicin (N), and 29 received ticarcillin and amikacin (A). Two patients receiving T & G (6.2%), one patient receiving T & N (3.4%), and one patient receiving T & A (3.4%) developed auditory toxicity with bilateral decreases of at least 20 db at one or more frequencies. Pharmacists were well received by the patients and successfully obtained and interpreted pre- and post-aminoglycoside audiograms.		

The objective of this study is to assess the relative incidence of auditory toxicity among three different aminoglycosides (gentamicin, netilmicin, and amikacin), each administered in combination with ticarcillin. It is our intention to study the monitoring function as performed by a pharmacist as well as to present the results of the auditory toxicity.

Ototoxicity, both auditory and vestibular, secondary to aminoglycoside antibiotics, has previously been reported with gentamicin and amikacin. Ninety patients received one of these combinations of empiric antibiotic therapy. Baseline and serial audiograms were obtained on these patients. There was no difference among the three groups in the average duration of aminoglycoside exposure, age, or underlying disease. Two patients receiving ticarcillin and gentamicin (6.2%), one patient receiving ticarcillin and netilmicin (3.4%), and one patient receiving ticarcillin and amikacin (3.4%) developed auditory toxicity with bilateral decreases of at least 20 db at one or more frequencies. None of these five patients were receiving other drugs which have been associated with ototoxicity during antibiotic therapy. Serum creatinines were monitored at least every other day with dosage changes made at the first sign of rising creatinine. None of the patients with ototoxicity demonstrated nephrotoxicity.

Publications:

1. Bender, J.F., Fortner, C.L., Schimpff, S.C., Grove, W.R., Hahn, D., Love, L., and Wiernik, P.H., Comparative Auditory Toxicity of Aminoglycoside Antibiotics in Leukopenic Patients. *Amer. J. Hosp. Pharmacy*, 36:1083-1087, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09112-01 COB																								
PERIOD COVERED October 1, 1979 to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) An Evaluation of the Effect of Chemotherapy and Oral Antibiotics on Gastrointestinal Function																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width:100%; border: none;"> <tr> <td style="width:35%;">PI: William R. Grove, B.S.</td> <td style="width:45%;">Assistant Director, Clinical Research Pharmacy Section</td> <td style="width:20%; text-align: right;">COB-NCI</td> </tr> <tr> <td>Clarence L. Fortner, M.S.</td> <td>Head, Clinical Research Pharmacy Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Stephen C. Schimpff, M.D.</td> <td>Head, Infections Research Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Kathryn A. Newman, R.N.</td> <td>Infections Control Nurse</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>OTHER: Peter P. Lamy, Ph.D.</td> <td>Professor, School of Pharmacy University of Maryland</td> <td></td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Peter H. Wiernik, M.D.</td> <td>Chief, Clinical Oncology Branch</td> <td style="text-align: right;">COB-NCI</td> </tr> </table>			PI: William R. Grove, B.S.	Assistant Director, Clinical Research Pharmacy Section	COB-NCI	Clarence L. Fortner, M.S.	Head, Clinical Research Pharmacy Section	COB-NCI	Stephen C. Schimpff, M.D.	Head, Infections Research Section	COB-NCI	Kathryn A. Newman, R.N.	Infections Control Nurse	COB-NCI				OTHER: Peter P. Lamy, Ph.D.	Professor, School of Pharmacy University of Maryland					Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI
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OTHER: Peter P. Lamy, Ph.D.	Professor, School of Pharmacy University of Maryland																									
Peter H. Wiernik, M.D.	Chief, Clinical Oncology Branch	COB-NCI																								
COOPERATING UNITS (if any) University of Maryland School of Pharmacy, Baltimore, MD																										
LAB/BRANCH Clinical Oncology Branch																										
SECTION Clinical Research Pharmacy Section																										
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201																										
TOTAL MANYEARS: .6	PROFESSIONAL: .6	OTHER:																								
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this study is to evaluate the effect of <u>chemotherapy</u> and <u>oral antibiotics</u> on <u>gastrointestinal function</u> in a population of patients with <u>acute non-lymphocytic leukemia</u> in remission. Eligible patients are those patients receiving the chemotherapy combination of <u>thioguanine</u> and <u>cytosine arabinoside</u> for maintenance chemotherapy and receiving any of the following combinations of oral antibiotics for infection prevention: gentamicin + nystatin; gentamicin + vancomycin + nystatin; nalidixic acid + nystatin; trimethoprim-sulfamethoxazole + nystatin. The following laboratory tests are performed as a baseline, repeated at the end of the chemotherapy course and again while on oral antibiotics: serum carotene, serum Vitamin A, serum folic acid, serum D-xylose (2 hr) and urine D-xylose (5 hr). Sixteen patients have been entered on study. Seven patients have completed the sequence of laboratory tests. Eight patients failed to complete the tests, mostly due to the need for hospitalization to receive systemic empiric antibiotics. One patient remains on study. Additional patients will be entered on the study as they begin their maintenance courses of chemotherapy. It is too early to evaluate the data.																										

The objective of this study is to evaluate the effect of chemotherapy and oral antibiotics on gastrointestinal function in a population of patients with acute non-lymphocytic leukemia in remission.

Eligible patients are those patients receiving the chemotherapy combination of thioguanine and cytosine arabinoside for maintenance chemotherapy and receiving any of the following combinations of oral antibiotics for infection prevention: gentamicin + nystatin; gentamicin + vancomycin + nystatin; nalidixic acid + nystatin; trimethoprim-sulfamethoxazole + nystatin.

The following laboratory tests are performed as a baseline, repeated at the end of the chemotherapy course and again while on oral antibiotics: serum carotene, serum Vitamin A, serum folic acid, serum D-xylose (2 hr) and urine D-xylose (5 hr).

Surveillance cultures of nose, gums and rectum are obtained prior to the use of oral antibiotics and repeated in one week, to verify compliance to the oral regimen. This microbiological information will be correlated to the results of the absorption studies to determine if there is a difference due to the various oral antibiotic regimens.

SMITHKLINE SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09121-01 COB										
PERIOD COVERED October 1, 1979 to September 30, 1980												
TITLE OF PROJECT (80 characters or less) The Prevention of Shaking Chills and Fever with Intravenous Meperidine or Hydrocortisone												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT												
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 55%;">Rebecca Finley, Pharm.D. Clinical Pharmacist</td> <td style="width: 30%; text-align: right;">COB NCI</td> </tr> <tr> <td rowspan="3">OTHER:</td> <td>Joseph Aisner, M.D. Head, Medical Oncology Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Clarence L. Fortner, M.S. Head, Clinical Research Pharmacy Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Peter H. Wiernik, M.D. Chief, Clinical Oncology Branch</td> <td style="text-align: right;">COB-NCI</td> </tr> </table>			PI:	Rebecca Finley, Pharm.D. Clinical Pharmacist	COB NCI	OTHER:	Joseph Aisner, M.D. Head, Medical Oncology Section	COB-NCI	Clarence L. Fortner, M.S. Head, Clinical Research Pharmacy Section	COB-NCI	Peter H. Wiernik, M.D. Chief, Clinical Oncology Branch	COB-NCI
PI:	Rebecca Finley, Pharm.D. Clinical Pharmacist	COB NCI										
OTHER:	Joseph Aisner, M.D. Head, Medical Oncology Section	COB-NCI										
	Clarence L. Fortner, M.S. Head, Clinical Research Pharmacy Section	COB-NCI										
	Peter H. Wiernik, M.D. Chief, Clinical Oncology Branch	COB-NCI										
COOPERATING UNITS (if any) None												
LAB/BRANCH Clinical Oncology Branch												
SECTION Clinical Research Pharmacy Section												
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201												
TOTAL MANYEARS: .5	PROFESSIONAL: .5	OTHER: .										
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords)												
The objective of this study is to evaluate and compare the efficacies of intravenous Meperidine and Hydrocortisone in preventing <u>shaking chills</u> associated with Amphotericin therapy. Patients are randomized to receive either Meperidine or Hydrocortisone by IV push prior to the infusion, or as an addition to the infusion. The occurrence of shaking chills and the time interval between the beginning of the amphotericin infusion and the chill are being evaluated.												

This is a double-blind randomized trial designed to evaluate and compare the efficacies of intravenous Meperidine and Hydrocortisone in the prevention of shaking chills in patients receiving amphotericin therapy. In addition, the route of administration (IV push vs addition to infusion bottle) is also being evaluated. Patients are eligible for the study if they have previously experienced shaking chills with amphotericin therapy.

Patients receiving Amphotericin therapy are randomized to receive one of the following randomizations: 1) 50 mg. of Meperidine, IV push, 5 min. before the amphotericin infusion; 2) 25 mg. of Hydrocortisone, IV push, 5 min. before the amphotericin; 3) 50 mg of Meperidine added to the amphotericin infusion, or 4) 25 mg of Hydrocortisone added to the amphotericin infusion.

Criteria which is being evaluated includes, the occurrence of shaking chills, the time interval between the beginning of the infusion to the onset of the shaking chills and adverse reactions related to any of the randomizations. To date, three patients have been entered on this double-blind study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09122-01 COB																		
PERIOD COVERED October 1, 1979 to September 30, 1980																				
TITLE OF PROJECT (80 characters or less) Amikacin Dosing Program																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Rebecca Finley, Pharm.D.</td> <td style="width: 33%;">Clinical Pharmacist, Clinical Research Pharmacy Section</td> <td style="width: 33%; text-align: right;">COB-NCI</td> </tr> <tr> <td colspan="3">OTHERS: James Wade, M.D.</td> </tr> <tr> <td></td> <td>Senior Investigator, Infections Research Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Ellis Caplan, M.D.</td> <td>Clinical Assoc. Professor</td> <td></td> </tr> <tr> <td>Stephen C. Schimpff, M.D.</td> <td>Head, Infections Research Section</td> <td style="text-align: right;">COB-NCI</td> </tr> <tr> <td>Clarence L. Fortner, B.S.</td> <td>Head, Clinical Research Pharmacy Section</td> <td style="text-align: right;">COB-NCI</td> </tr> </table>			PI: Rebecca Finley, Pharm.D.	Clinical Pharmacist, Clinical Research Pharmacy Section	COB-NCI	OTHERS: James Wade, M.D.				Senior Investigator, Infections Research Section	COB-NCI	Ellis Caplan, M.D.	Clinical Assoc. Professor		Stephen C. Schimpff, M.D.	Head, Infections Research Section	COB-NCI	Clarence L. Fortner, B.S.	Head, Clinical Research Pharmacy Section	COB-NCI
PI: Rebecca Finley, Pharm.D.	Clinical Pharmacist, Clinical Research Pharmacy Section	COB-NCI																		
OTHERS: James Wade, M.D.																				
	Senior Investigator, Infections Research Section	COB-NCI																		
Ellis Caplan, M.D.	Clinical Assoc. Professor																			
Stephen C. Schimpff, M.D.	Head, Infections Research Section	COB-NCI																		
Clarence L. Fortner, B.S.	Head, Clinical Research Pharmacy Section	COB-NCI																		
COOPERATING UNITS (if any) University of Maryland Infectious Disease Division																				
LAB/BRANCH Clinical Oncology Branch																				
SECTION Clinical Research Pharmacy Section																				
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201																				
TOTAL MANYEARS: .3	PROFESSIONAL: .3	OTHER: 																		
CHECK APPROPRIATE BOX(ES) ☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this study is to establish the predictability of a programmable TI-59 <u>Calculator program</u> in establishing amikacin doses based on lean body weight, estimated creatinine clearance and desired <u>serum peak</u> and <u>trough levels</u> . Steady state serum peak and trough levels are being measured using a <u>radioimmunoassay</u> technique. The measured levels are being used to calculate the elimination rate constant and volume of distribution. If necessary, the maintenance dose is adjusted using the calculated elimination rate constant, volume of distribution and desired serum levels. Serum creatinine and BUN are monitored to determine changing renal function.																				

A programmable TI-59 calculator is being utilized to establish appropriate amikacin dosages for patients receiving this aminoglycoside in combination with either ticarcillin or Moxalactam. The initial loading dose, maintenance dose and dosing interval are calculated using the patient's lean bodyweight, estimated creatinine clearance and desired steady state serum peak and trough levels of 25 mcg/ml and 5 to 8 mcg/ml, respectively. After the serum levels of amikacin have reached steady state, the serum peak and trough levels are measured using a radioimmune assay technique. The elimination rate constant and the volume of distribution are calculated based on the measured serum levels, and the maintenance dose is adjusted to the desired serum levels using these values, if necessary. Repeat serum peak and trough levels are measured every 3 to 5 days to assure that the patient is maintaining the desired therapeutic levels of amikacin.

At the conclusion of the study, the predictability of the calculator program in establishing appropriate doses will be evaluated.

Throughout the course of amikacin therapy, the serum creatinine and blood urea nitrogen are monitored on a daily basis in order to determine changes in renal function which might effect amikacin serum levels or possibly indicate amikacin toxicity.

SUMMARY
Laboratory of Clinical Biochemistry
October 1, 1979 to September 30, 1980

Since moving to the University of Maryland, the major administrative efforts in the Laboratory of Clinical Biochemistry have been to continue to build and to solidify collaborative research efforts between the Laboratory of Clinical Biochemistry and the neighboring medical and basic research groups. With the Clinical Oncology Branch the interaction in clinical pharmacology has expanded and protocols are ongoing for the investigation of clinical pharmacology in Phase I and Phase II studies. In addition, an emphasis has been placed on the investigation of response modifiers such as hyperthermia on the pharmacology of agents. A number of joint seminars, teaching sessions, and meetings are carried out routinely between the Laboratory of Clinical Biochemistry and the cooperating groups.

With departments in the University of Maryland School of Medicine such as the Medicine Department, Biochemistry Department, and Radiobiology, we have a number of collaborative efforts ongoing. In addition, our interaction with the School of Pharmacy is quite rewarding especially in view of the back up that the School provides from its Department of Pharmacology and Department of Medicinal Chemistry.

Our research program in biochemical pharmacology has been oriented for a comprehensive study and understanding of drug, free radical pharmacology. Although we made observations concerning the existence of these species and have predicted that a site-specific free radical should exist, we have oriented our research effort to delineate and characterize the biochemical mechanisms of formation of drug, free radicals. We have shown that flavoenzymes are primarily responsible for the catalytic production of drug, free radicals from quinone and quinone imine containing anticancer agents. Only flavoenzymes capable of single electron transport catalyze the production of free radicals and the reductive cleavage of the anthracycline antibiotics adriamycin and daunorubicin. Flavoproteins which catalyze two electron reductions are not active in these systems. We have established the biochemical machinery for this important pharmacologic event. In view of our work with quinone containing antibiotics, we have now shown that the quinone imine containing antibiotic, actinomycin D is reduced enzymatically to a free radical state. Through the use of model phenoxazone compounds and hyperfine EPR studies, we have predicted the electron residency in these ring systems so that we can locate reactive sites of the actinomycin D molecule.

In the study of human metabolic pathways for chemotherapeutic agents, we have purified from human liver the daunorubicin pH 6.0 reductase; and we have shown this enzyme is dependent on NADPH as a cofactor, has a molecular weight of 31,000, and consists of a single peptide unit. We have shown this to be closely related with a family of aldehyde reductases. The pH 6.0 reductase is the first ketone reductase that has been purified to homogeneity.

The problem of metal-anthracycline interaction and complexes has plagued the scientific understanding of anthracycline cardiotoxicity and anthracycline action for several years. Since the reported iron-adriamycin mixture, "quelamycin," was described by Dr. Gosavetz, claims concerning quelamycin structure and action have been in question. We have shown selective adriamycin tissue concentrations are decreased in animals injected with iron-adriamycin complexes which may be the cause of decreased

cardiac toxicity from this mixture. However, we find that the change in activity of the adriamycin results from a change in the pharmacologic disposition of the material because of a physical-chemical difference from adriamycin. Iron-adriamycin forms solid microaggregates which dramatically affect the distribution and disposition of the adriamycin causing it to collect in lung capillaries when administered intravenously. This creates a depot from which the adriamycin can leech out slowly when dissociated from the iron. To fortify our continuing clinical pharmacology effort for the study of anthracyclines such as newly developed ones as eclacinomycin A, we have developed a new high pressure liquid chromatographic system which allows for the simultaneous separation of adriamycin and its metabolites or daunorubicin and all its metabolites in one rapid and sensitive assay. The sensitivity depends on our modification of a newly developed fluorescence monitor which is selective for the anthracycline-type drugs.

In our study of the cellular control mechanisms which affect cell growth and differentiation, we have shown that deoxycytidine is a more effective inhibitor of cell growth and colony formation of the human leukemia HL-60 and K-562 than thymidine. Both of these nucleosides inhibit tritiated nucleoside incorporation; but the action of deoxycytidine is directly through the effect on the intracellular nucleotide pool. Deoxyguanosine is also interesting in that in combination with cytosine arabinoside, this nucleoside is synergistic in cell kill and stimulates incorporation of cytosine arabinoside into cellular DNA. These observations with natural nucleosides may be important in the use as response modifiers with known chemotherapeutic agents.

A study of untreated patients with acute myelocytic leukemia show that they possess both in their marrow and in their peripheral blood a "herpes viral like" thymidine kinase at a very elevated level which decreases when the leukemia is in remission. Tailored efforts at the inhibition of this enzyme through selective chemotherapeutic usage is being attempted to evaluate this as a chemotherapeutic method.

In a study of our patients with acute nonlymphocytic leukemia we find that they as a group have less natural killer cell activity against the HL-60 tissue culture line. Immunotherapy of these patients with neuraminidase treatment did not alter the natural killer cell activities in the patients; and the natural killer cell activity appears to correlate well with the disease status in the bone marrow.

A study of the survival time of AKR mice transplanted with syngeneic lymphoma cells was increased by three weekly injections of *C. parvum*. This is correlated with the ability of *C. parvum* to activate peripheral blood monocytes but not peritoneal macrophages. The local suppression of transplanted lymphoma by macrophages did not prolong the survival of the mice if the blood monocytes were not activated. We have also developed clones of AKR lymphoma which are resistant to macrophage suppression.

In our continuing and amplified studies of clinical pharmacology, the most important projects have been the investigation of the effect of response modifiers on the clinical pharmacology of anticancer agents. Hyperthermia is one response modifier that dramatically alters the pharmacokinetics of cyclophosphamide; and we have been able to understand more of the normal pharmacology of this agent through this parallel study of patients under hyperthermia and normothermia receiving this drug. These studies have been extended into laboratory animals from this initial clinical observation. We also find that dimethylsulfoxide which is synergistic in animals with cyclophosphamide does not alter the pharmacokinetics of cyclophosphamide nor does it alter the clinical response of patients with epidermoid carcinoma. Daunorubicin in combination with

certain other drugs leads to elevated plasma concentrations of daunorubicin, and altered rates of excretion of daunorubicin and its metabolites. We feel that this is an important observation for the warning of potential increased toxicity with these agents.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06012-10 LCB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Biochemical Pharmacology of Antineoplastic and Other Agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	N.R. Bachur R.L. Felsted H. Nakazawa S. Pan P. L. Gutierrez M.V. Gee R.D. Friedman F.E. Chou	Chief Research Chemist Visiting Fellow IPA IPA Chemist Chemist Visiting Scientist
		LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Biochemistry		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland		
TOTAL MANYEARS: 5.3	PROFESSIONAL: 4.5	OTHER: 0.8
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Actinomycin D can be reduced <u>enzymatically</u> , <u>chemically</u> or <u>electrochemically</u> to a free radical state. Absorption and <u>electron spin resonance</u> spectra of actinomycin D and its <u>phenoxazone</u> analogs show that a one electron reduction produces the free radical and yields <u>hyperfine structure</u> for prediction of <u>electron residency</u> . The <u>flavoenzymes</u> , NADPH cytochrome P-450 reductase, xanthine oxidase, NADPH cytochrome C reductase, <u>nitrate reductase</u> , and <u>lipoamide dehydrogenase</u> catalyze reductive cleavage of the anthracyclines and free radical formation. Human daunorubicin pH 6.0 reductase is purified and classified as a ketone reductase.		

Project Description:

In our studies on mechanism of pharmacological action of antitumor agents, we observed that some of them are biochemically activated to a free radical form. Actinomycin appeared capable of reduction to a free radical. Therefore we investigated the free radical formation of actinomycin D and its analogs by chemical, electrochemical, and biological reactions. Chemical reduction with sodium borohydride changed the absorption spectra of actinomycin D, 2-amino-3-phenoxazone and 1,2,4-trichloro-7-nitro-phenoxazone in the visible region and also produced evidence of a free radical by electron spin resonance spectra in dimethyl sulfoxide. The DC polarograms of these compounds in acetonitrile showed two reduction waves. When reduced at a potential corresponding to their first half wave potential, a one-electron reduction product formed yielding a free radical state that was confirmed by spin resonance spectra. Under anaerobic conditions, rat liver microsomes and NADPH cytochrome P450 reductase catalyzed an NADPH dependent oxygen consumption with actinomycin D and a free radical was produced in the reaction. These results clearly show that actinomycin D can be readily reduced to a free radical state. The free radical form of actinomycin D may be a pharmacologically active form of this tumor agent for DNA interaction and subsequent toxicity.

Several flavo-enzymes are compared for their abilities to catalyze reductive glycosidic cleavage of anthracycline antibiotics. NADPH cytochrome P450 reductase, xanthine oxidase, nitrate reductase, NADH cytochrome c reductase and lipoamide dehydrogenase catalyzed with decreasing specific activity the reductive glycosidic cleavage of daunorubicin. Glutathione reductases, L-amino acid oxidase, D-amino acid oxidase, D-glucose oxidase, and aldehyde reductase were inactive. Those flavo-enzymes which catalyze glycosidic cleavage all possess single electron transfer systems. The formation and interconversion of free radical anions of anthracycline antibiotics and their important metabolites were investigated with xanthine oxidase and NADPH cytochrome P-450 reductase. Electron paramagnetic resonance spectra indicated that the parent antibiotics daunorubicin and adriamycin, and metabolites including aglycones, deoxyaglycones, and alcohol derivatives all generated free radicals through the catalysis of these two enzymes under anaerobiosis. In time, a second electron paramagnetic resonance signal emerged for daunorubicin and adriamycin while the initial signal disappeared. Both initial spectra had a g value of 2.0036. The fully developed second signal of these antibiotic free radicals showed the same parameters as the electron paramagnetic resonance spectrum of their deoxyaglycones, asymmetry and g anisotropy ($g_{\parallel} = 2.0052$ and $g_{\perp} = 2.0023$).

The metabolism of the anticancer agents, adriamycin and daunorubicin, is initiated by cytoplasmic carbonyl reductases. These reductases produce, in the presence of the cofactor NADPH, the respective metabolites adriamycinol and daunorubicinol. Several daunorubicin reductases have been purified from human liver by ammonium sulfate precipitation, gel filtration on Sephadex G100, affinity chromatography and NADP⁺-agarose, and ion exchange chromatography on DEAE C1-6B Sepharose. All the reductases were copurified until the last ion exchange step where they were resolved by a shallow salt gradient. The reductases were distinguished primarily by their pH optima. Two enzymes reduce daunorubicin with an optimum of pH 6.0 whereas one enzyme reduces daunorubicin with an optimum of pH 8.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of daunorubicin 6.0 and daunorubicin 8.5 reductases shows one major protein band (greater than 90% of stain) for each enzyme with molecular weights of 31,000 and 36,000 daltons, respectively. Analogous

enzymes were purified from rabbit liver with similar results. The daunorubicin 8.5 reductase is similar to rat liver daunorubicin reductase which was previously identified as an aldehyde reductase. In contrast, the daunorubicin 6.0 reductases display a broader ketone specificity and are ketone reductases. The enzyme structure and substrate specificities are compared in order to assess the relationships of these carbonyl reductases.

Publications:

Ahmed, N.K., Felsted, R.L., and Bachur, N.R.: Comparison and characterization of mammalian xenobiotic ketone reductases. *J. Pharmacol. Exp. Ther.* 209: 12-19, 1979.

Bachur, N.R. Gordon, S.L., Gee, M.V., and Kon, H.: NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proc. Natl. Acad. Sci USA*, 76: 954-957, 1979.

Caspary, W.J., Nisiak, C., Lanzo, D.A., Friedman, R., and Bachur, N.R.: Bleomycin A₂: A ferrous oxidase. *Mol. Pharmacol.* 16: 256-260, 1979.

Felsted, R.L. and Bachur, N.R.: Ketone reductases in enzymatic basis of detoxication (Ed. W.B. Jacoby) Academic Press, New York, Vol. 1, pp. 281-293, 1980.

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cellular Control Mechanisms Affecting Cell Growth and Differentiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N.R. Bachur	Chief	LCB, NCI
OTHER:	R.L. Felsted	Research Chemist	LCB, NCI
	A. Schrecker	Research Chemist	LCB, NCI
	D.D. Ross	Clinical Associate	LCB, NCI
	S.A. Akman	Clinical Associate	LCB, NCI

COOPERATING UNITS (if any)

Department of Biochemistry Emory University, Atlanta, Georgia, COB, BCRP

LAB/BRANCH

Laboratory of Clinical Biochemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Baltimore, Maryland 21201

TOTAL MANYEARS:

2.7

PROFESSIONAL:

2.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS☒ (b) HUMAN TISSUES☐ (c) NEITHER☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Deoxycytidine is a more effective inhibitor of cell growth and colony formation of the human leukemia HL-60 and K-562 than is thymidine and inhibits tritiated nucleoside incorporation. This action is through a direct effect on intracellular nucleotide pool size. In combination with cytosine arabinoside, deoxyguanosine effects the cell kill and incorporation of cytosine arabinoside into cellular DNA. Patients with acute Myelocytic leukemia show evidence of herpes virus like thymidine kinase in both their bone marrow and peripheral blood.

Project Description:

The potential cancer chemotherapeutic effect of thymidine led to our investigation of the mechanism of cell growth inhibition caused by this and the other deoxyribonucleosides, namely deoxyguanosine, deoxyadenosine, and deoxycytidine. Growth of human leukemic cell lines K-562 and HL-60 was measured by cloning in soft agar. Cells were cultured in RPMI-1640 medium with 10% (for K-562) and 20% (for HL-60) fetal calf serum. Final agar concentration was 0.3%. Colonies were counted after 14 days incubation. The concentrations of nucleoside causing 50% inhibition of HL-60 colony growth (IC_{50}) were: deoxycytidine greater than 10mM; deoxyadenosine, 1mM; thymidine, 2-5mM; deoxyguanosine, 0.08mM. The IC_{50} for K-562 colony growth was greater than 10mM for deoxycytidine, 1.6mM for deoxyadenosine, 0.33mM for thymidine, and 0.1mM for deoxyguanosine. Investigating the effects of each nucleoside on the incorporation of the other three ^3H -labeled nucleosides into acid insoluble material, we preincubated cells in liquid suspension culture in the presence of unlabeled nucleoside for 24 hours, after which a 60 minute pulse of a different ^3H -nucleoside was given. Preincubation with 0.5mM thymidine caused about a 3 to 4-fold increased incorporation of ^3H -deoxycytidine into acid insoluble material in HL-60 and K-562. Preincubation with 0.1mM deoxyguanosine caused 2-3 fold enhancement of ^3H -deoxycytidine incorporation in K-562 and HL-60 cells. Neither preincubation with deoxycytidine nor deoxyadenosine caused enhanced incorporation of any other labeled nucleoside, nor was incorporation of ^3H -deoxycytidine or ^3H -deoxyguanosine enhanced by preincubation with thymidine. We interpret the enhanced ^3H -deoxycytidine incorporation into acid insoluble material to be due to a decrease in the endogenous cellular pool size of deoxycytidine phosphates caused by preincubation with thymidine or deoxyguanosine. To test this hypothesis, we measured the effects of a 16-hour incubation with 0.75mM thymidine or deoxyguanosine on the nucleotide triphosphate pool sizes of HL-60 cells. The reduction of intracellular pools of deoxycytidine caused by 0.75mM thymidine or deoxyguanosine was 74 and 80% respectively. Moreover, 1mM deoxycytidine completely reversed the inhibitory effect of 10mM thymidine on HL-60 cell growth in liquid suspension culture. The "rescuing" effect of deoxycytidine was less marked for HL-60 colony growth; however, 1mM deoxycytidine increased the IC_{50} of HL-60 colonies to 8mM for thymidine and to 0.23mM for deoxyguanosine. We conclude that of the deoxynucleosides tested, the order of inhibition to cell cloning was deoxyguanosine greater than thymidine equal to deoxyadenosine greater than deoxycytidine. The mechanism of cell growth inhibition by thymidine and deoxyguanosine may involve reduction in deoxycytidine nucleotide pools. In vivo testing of the anti-tumor effects of deoxyguanosine are currently in progress in our laboratory.

We found that deoxyguanosine is a more potent inhibitor of HL-60 soft agar cloning than thymidine (IC_{50} deoxyguanosine = 500 μ M; IC_{50} thymidine = 5000 μ M). We investigated whether deoxyguanosine like thymidine, augmented the inhibition of HL-60 cloning by ara-C. Addition of lesser or equal to 10 nM ara-C to cloning medium had no effect on HL-60 cloning efficiency, nor did lesser than or equal to 100 μ M deoxyguanosine. However, concomitant addition of 5 nM ara-C plus 50 μ M deoxyguanosine reduced cloning efficiency by 40%, 5 nM ara-C plus 100 μ M deoxyguanosine by 60%, indicating synergy. We investigated whether this synergy could be explained by altered ara-C uptake, phosphorylation, or utilization by DNA polymerase. HL-60 cells in early log phase liquid suspension culture were incubated for 3 hours in the presence of 0.5 nM ^3H ara-C (26 Ci/mmol) plus or minus deoxyguanosine. After incubation, cells were iced and washed twice with ice-cold excess unlabeled ara-C. Trichloroacetic acid precipitable, total acid soluble, and total cellular ^3H tritium were

determined. To determine ara-C and ara-C triphosphate pool size, the acid soluble fraction was analyzed by anion exchange thin layer chromatography. Cellular ara-C uptake was reduced to 32-55% of control by 500 μ M deoxyguanosine. Deoxyguanosine reduced ara-C triphosphate pool size in proportion to the reduction in acid soluble tritium. At any concentration of deoxyguanosine, the ara-C triphosphate fraction of acid soluble tritium remained about 70%. 500 μ M deoxyguanosine reduced trichloroacetic acid precipitable radioactivity by 0-50%; however, it increased the percentage of total cellular ara-C incorporated into nucleic acid by 158-225%. In other experiments, 0.5 nM ara-C plus 500 μ M deoxyguanosine caused a 60% decrease in tritiated thymidine incorporation into trichloroacetic acid precipitable material. These data suggest that deoxyguanosine reduces HL-60 cellular uptake of ara-C, but augments utilization of ara-C triphosphate by DNA polymerase.

The finding by Groth, et al, (Fed, Proc, 37, 1543, 1978) of herpes viral-like thymidine kinase activity in the K-562 leukemic cell line prompted our search for similar activity in blasts from acute myelogenous leukemia patients. Our study groups consisted of acute myelogenous leukemia untreated (11), acute myelogenous leukemia remission (14), and control (normal volunteer) (9). Cell free extracts of bone marrow mononuclear cells (isolated on a Ficoll-Hypaque gradient) were tested for enzyme activity. Herpes viral-like thymidine kinase was assayed by the method of Summers, et al. (J. Virol. 24, 314, 1977); total cellular thymidine kinase was determined with tritiated-thymidine and extraction of phosphorylated product on DEAE paper. Our results are summarized as follows ($\pm$ S.E.M.):

Study Group	HVL-TK	Total TK
AML untreated	100 $\pm$ 10	916 $\pm$ 218
AML remission	27 $\pm$ 3	159 $\pm$ 43
Control	21 $\pm$ 1	286 $\pm$ 34

Enzyme activity is expressed as femtomoles of product/ 10^6 cells/min. For acute myelogenous leukemia untreated, herpes viral-like thymidine kinase activity showed a five-fold increment above baseline activity displayed by control ($p < .001$), and these levels were comparable to those found in K-562. Total thymidine kinase was also elevated in the acute myelogenous leukemia untreated group, but herpes viral-like thymidine kinase accounted for a greater percentage of total thymidine kinase in this group (average of 11 individual percentages=19.9%, range 5.4-73.3) than in control (average=8.1%, range 3.5-13.6) ($p < .001$). Slight elevation of herpes viral-like thymidine kinase activity was found in the acute myelogenous leukemia remission group, however, this was significant from control ($p < .001$). We conclude that there is elevated activity of an enzyme with properties similar to a herpes virus gene product in cells from patients with acute myelogenous leukemia.

Publications:

Egorin, M.J., Bachur, S.M., Felsted, R.L., Leavitt, R.D., and Bachur, N.R.: Phaseolus vulgaris isolectin binding to human erythrocytes. J. Biol. Chem. 254: 894-898, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06016-10 LCB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Pharmacodynamics of Cancer Chemotherapeutic Agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	N. R. Bachur M. J. Egorin P. A. Andrews F. E. Chou R. D. Friedman R. Clawson A. Pollak	Chief, Expert Lab. Scientist Visiting Scientist Chemist Lab. Scientist Lab. Scientist LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Biochemistry		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 0.81	OTHER: 1.2
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<u>Metal adriamycin complexes</u> show interesting differences from the parent adriamycin in basic pharmacology. <u>Iron adriamycin</u> forms microaggregates on preparation which dramatically affect the distribution and disposition of the <u>adriamycin</u> such as lowering the <u>cardiac content</u> of adriamycin. We have developed a <u>high pressure liquid chromatographic method</u> and a <u>new fluorescence detector</u> which allow the simultaneous separation of <u>adriamycin and all its metabolites</u> or daunorubicin and all its metabolites.		

Project Description:

Previous studies have reported antitumor activity and reduced cardiotoxicity for a putative 3:1 complex of iron (Fe):adriamycin (ADR). We have studied the tissue distribution and metabolism of a wide variety of freshly prepared and lyophilized Fe:ADR preparations after administration to BALB/C mice (16 mg/kg ADR i.v.). Tissue concentrations of adriamycin given without iron were initially highest in kidney and liver, and adriamycin fluorescence was lost from all tissues except spleen with a $t_{1/2}$ of 15-20 hours. Adriamycin remained the major fluorescent species in liver and kidney from 1/2 - 72 hours after treatment. Freshly prepared 1:1 Fe:ADR behaved similarly to adriamycin except for a longer tissue $t_{1/2}$ of approximately 36 hours. The tissue distribution of freshly prepared 2:1 and 3:1 Fe:ADR was very different from that of adriamycin without iron: the lung containing the highest concentrations of adriamycin fluorescence. Renal and hepatic metabolism of adriamycin was not altered by the iron in any of the fresh Fe:ADR preparations. The tissue distribution of 1:1 Fe:ADR that had been aged for 48 or 96 hours was similar to that of fresh 2:1 and 3:1 Fe:ADR rather than adriamycin or fresh 1:1 Fe:ADR. When lyophilized Fe:ADR preparations were reconstituted and administered, the 1/2 hour tissue distribution of 0.1:1, 0.2:1, 0.25:1, and 0.33:1 Fe:ADR was the same as adriamycin alone, but 0.5:1, 1:1, 2:1, and 3:1 Fe:ADR were all accumulated primarily in the lung. Fe:ADR preparations which were accumulated in the lung, produced lower cardiac concentrations of adriamycin fluorescence than did Fe:ADR preparations which were distributed primarily to the liver and kidney. Physical-chemical studies confirm the production of microaggregated Fe:ADR complexes, and light microscopy allows visualization and sizing of these aggregates. We feel that the observed dramatic alteration in tissue distribution reflects trapping of these Fe:ADR aggregates in the lung with subsequent slow release into the systemic circulation.

Although numerous high pressure liquid chromatographic systems for the separation of anthracyclines in plasma have recently been described, none have characterized all the known human metabolites of adriamycin and daunorubicin. We used a Spectra Physics model 3500 high pressure liquid chromatograph fitted with a Bondapak-Phenyl 3.9 mm x 30 cm column. Adriamycin and daunorubicin metabolites were eluted with a linear gradient over 10 minutes from 85% pH 4.0, 0.1% (w/v) ammonium formate buffer, 15% tetrahydrofuran to 50% ammonium formate buffer, 50% tetrahydrofuran at a flow rate of 2.0 ml/min. Adriamycin and daunorubicin were extracted from 1.0 ml of human plasma with 2 ml chloroform: 2-propanol (1:1) with 50-70% efficiency and quantified against an internal standard (daunorubicin or adriamycinol respectively). The fluorescence detector consists of a modified fluorometer (Aminco) utilizing a G.E. F4T5 blue lamp primary filter, a 510 nm cutoff secondary filter, and a 90 μ l flow cell. Sensitivities of 1 picomole/ml with standard solutions and 30 picomole/ml from plasma extracts were obtained. The following adriamycin metabolites were separated (k'): 4-O-glucuronide (2.75), 4-O-sulfate (4.00), adriamycinol (5.5), A2a (6.0), adriamycin (6.63), dA2a (7.25), A1a (7.25), dA1a (8.38), demethyl-dA2a (9.6). Daunorubicin metabolites separated (k'): 4-O-glucuronide (4.25), daunorubicinol (6.75), D2a (7.25), daunorubicin (7.88), dD2a (8.38), D1a (8.88), dD1a (9.63), demethyl dD2a (11.0). Representative plasma from patients contained adriamycin and adriamycinol with $T_{1/2}$ approximately eight hours each; daunorubicin and daunorubicinol with $T_{1/2}$ 3.4 and 12.9 hours respectively. D2a was also found in plasma. We recovered A1, A2, and 4-O-sulfate metabolites from urine. This method offers the advantage of a simultaneous separation of adriamycin and eight metabolites, or daunorubicin and seven metabolites, in one rapid and sensitive assay.

Publications:

Reich, S.D., Steinberg, F., Bachur, N.R., Riggs, C.E., Goebel, R., and Berman, M.: Mathematical model for adriamycin (doxorubicin) pharmacokinetics. J. Cancer. Chemother. Pharmacology 3: 125-131, 1979.

Ostrow, S., Egorin, M., Aisner, J., Bachur, N., and Wiernik, P.H.: High dose cis-diammine dichloroplatinum therapy in patients with advanced breast cancer: Pharmacokinetics, toxicity, and therapeutic efficacy. Cancer Clin. Trials 3: 23-27, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06241-10 LCB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Clinical Pharmacology of Antineoplastic and Other Drugs		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: OTHER:	N. R. Bachur M. J. Egorin F. E. Chou C. E. Riggs P. A. Andrews B. Fox S. S. Ostrow D. Van Echo S. D. Markus M. Whitacre D. Brenner J. Aisner P. H. Wiernik L. Grochow M. Colvin	Chief Expert Visiting Scientist Clinical Associate Lab. Scientist Lab. Scientist Clinical Associate Senior Investigator Nurse Nurse Clinical Associate Senior Investigator Acting Associate Director Investigator Investigator LCB, NCI LCB, NCI LCB, NCI LCB, NCI LCB, NCI COB, NCI COB, NCI COB, NCI COB, NCI COB, NCI COB, NCI COB, NCI COB, NCI ECRP, NCI J.H.H. Oncology Center J.H.H. Oncology Center
COOPERATING UNITS (if any) Clinical Oncology Branch, Baltimore Cancer Research Program NCI Johns Hopkins Oncology Center		
LAB/BRANCH Laboratory of Clinical Biochemistry		
SECTION		
INSTITUTE AND LOCATION NCI, NIH Baltimore, Maryland 21201		
TOTAL MANYEARS: 4.5	PROFESSIONAL: 2.5	OTHER: 2.0
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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
We have initiated new research efforts in our <u>clinical pharmacology</u> project for the study of <u>response modifiers</u> on the clinical pharmacology of anticancer drugs. <u>Hyperthermia</u> dramatically alters the <u>pharmacokinetics of cyclophosphamide</u> and hopefully the clinical effects of this and other drugs. <u>Dimethylsulfoxide</u> neither alters the pharmacokinetics of cyclophosphamide nor the clinical response in patients with epidermoid carcinoma of the lung. <u>Dauorubicin administered in repetitive doses in combination chemotherapy</u> shows altered pharmacokinetics and disposition than that administered as single dosage.		

Project Description:

Since the observations from experimental cellular and animal chemotherapy that hyperthermia is frequently synergistic with anticancer drugs, clinical trials have developed for the evaluation of protocols combining whole body heat and chemotherapy. We have initiated a task force approach to the study of the clinical pharmacology of drugs during hyperthermia. In addition to the cooperative clinical studies, we are working on in vitro experimentation to help explain our clinical observations.

Synergism between cyclophosphamide and hyperthermia has been demonstrated in vitro. Eight patients with advanced cancer received whole body heat (40.6°C or 41.8°C) and cyclophosphamide. Four patients received 1 to 1.5 grams of cyclophosphamide infusion at normal temperature 37°C , 3 to 4 weeks prior to whole body heat. Terminal half-life, peak plasma cyclophosphamide levels, peak plasma alkylating activity, and 24 hour urinary excretion of cyclophosphamide and alkylating activity were measured at 37°C , whole body heat and when cyclophosphamide was given four hours following a two hour course of whole body heat and adriamycin.

There was a longer terminal half-life of cyclophosphamide during whole body heat and following whole body heat when compared to 37°C . The 24 hour urinary excretion of cyclophosphamide was increased four hours following whole body heat compared to 37°C . When compared to cyclophosphamide courses at 37°C , the 24 hour urinary excretion of alkylating activity was decreased following cyclophosphamide with whole body heat in one patient and cyclophosphamide given four hours after whole body heat in three patients. Whole body heat alters cyclophosphamide pharmacokinetics by increasing urinary excretion of cyclophosphamide and increasing terminal half-life of cyclophosphamide. We are initiating studies with other agents during human hyperthermic therapy.

Dimethylsulfoxide penetrates most cell barriers and increases tissue penetration of many pharmacologic agents. Dimethylsulfoxide and cyclophosphamide has led to acceleration of tumor shrinkage, delayed onset of leukemic meningitis and increased cyclophosphamide metabolite concentrations in blood and urine of animals. Five liters of 5% dimethylsulfoxide solution was given over three days orally along with cyclophosphamide 1.5 gm/m² by one hour I.V. infusion on the third day to nine patients with epidermoid carcinoma of the lung. Eight of the nine patients had received prior chemotherapy and/or radiation. Courses were repeated every three to four weeks. Toxicities from cyclophosphamide included nausea and vomiting in all patients, alopecia, nadir wbc 1000 cells/cu. mm in eight patients and nadir platelet count 100,000/cu. mm in three patients. There was no apparent dimethylsulfoxide toxicity. Pharmacokinetic studies of six courses in four patients showed mean peak plasma cyclophosphamide of $74.4 \pm 27.3 \mu\text{g/ml}$ ($\pm$ s.d.), mean plasma cyclophosphamide terminal half-life of 6.4 ± 4 hours, and mean peak plasma alkylating activity of $3.8 \pm 2.4 \text{ nmol/ml}$. During the first 24 hours, urinary excretion of unmetabolized cyclophosphamide and alkylating activity accounted for $2.3 \pm 1.0\%$ and $15.3 \pm 4.8\%$, respectively of the cyclophosphamide infused. Cerebrospinal fluid cyclophosphamide levels at four, five, and 24 hours after cyclophosphamide infusion in three patients were $29.4 \mu\text{g/cc}$, $36.6 \mu\text{g/cc}$, and $.6 \mu\text{g/cc}$ which were 43%, 85%, and 56% of concomitant plasma levels, respectively. Concomitant cerebrospinal fluid alkylating activities in these three patients were .08, .4, and 2.3 nm/cc respectively. No antitumor responses have been seen in the nine patients. Pharmacokinetic data of cyclophosphamide in these patients' blood, urine, and cerebrospinal fluid is similar to cyclophosphamide given

without dimethylsulfoxide reported in other series.

Daunorubicin is used now most frequently in combination drug regimens for remission induction in acute non-lymphocytic leukemia (ANLL). Previous studies report the pharmacokinetics of daunorubicin given only as a single agent. In 13 patients with ANLL, daunorubicin at a dosage of 75 mg/square meter of body surface area on day 1 was given in combination with cytosine arabinoside (75 mg/square meter body surface area bid x 5 days), 6-thioguanine (75 mg/square meter of body surface area bid x 5 days), and pyrimethamine (1 mg/kg/day x 5 days). Plasma samples were obtained for 48 hours after the daunorubicin dose. Parent drug and metabolite levels were determined by thin layer chromatography of chloroform-2-propanol extracts of plasma. Fluorescence analysis of acidified ethanol extracts of plasma gave total anthracycline concentration in each sample. Compared to drug levels previously reported in patients treated with daunorubicin alone (80 mg/square meter of body surface area), total anthracycline concentrations were higher in patients who received combination therapy. Half-times of disappearance in the terminal excretion phase were 29.3 hours for total fluorescence, 20.6 hours for daunorubicin, and 27.9 hours for the major metabolite, daunorubicinol, resembling those seen with a daunorubicin dosage of 180 mg/square meter of body surface area. Aglycone metabolites, which may be related to long-term toxicities seen with daunorubicin, were present in high concentrations, often higher than those of daunorubicin or daunorubicinol at 36 and 48 hours. It is concluded that combination of daunorubicin with certain drugs leads to elevated plasma concentrations and altered rates of excretion of daunorubicin and its metabolites, with potential for increased toxicity.

Publications:

Bachur, N.R.: Anthracycline antibiotic pharmacology and metabolism. *Cancer Treat. Rep.* 63: 817-820, 1979.

Egorin, M.J., Clawson, R.E., Cohen, J.L., Ross, L.A., and Bachur, N.R.: Cellular pharmacology of 7(R)-O-methylnogarol: A new anticancer agent. *J. Pharmacol. Exper. Therap.* 210: 229-236, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06992-03 LCB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Cell Mediated Immune Response in AKR Lymphoma		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: L. Hsu A. Pollak	Senior Investigator Laboratory Scientist	LCB, NCI LCB, NCI
COOPERATING UNITS (if any) COB, BCRP		
LAB/BRANCH Laboratory of Clinical Biochemistry		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The median survival time of <u>AKR mice</u> transplanted with syngeneic lymphoma cells was increased by three weekly <u>injections of C. parvum</u> prior to tumor transplant. The enhanced tumor resistance is correlated with the ability of C. parvum to activate the peripheral blood <u>monocytes</u>, but not the <u>peritoneal macrophage</u>. Local suppression of transplanted lymphoma by macrophages did not prolong the survival of mice if the blood monocytes were not activated. There is preliminary evidence suggesting that macrophage resistant clone(s) of AKR lymphoma exist. Patients with <u>acute nonlymphocytic leukemia</u> as a group had significantly less <u>natural killer cell</u> activities against <u>HL-60</u>, a <u>human progranulocytic leukemic cell line</u>, as compared to a group of normal individuals. The immunotherapy with neuraminidase treated allogeneic myeloblasts did not alter the natural killer cell activities in the patients. The natural killer cell activities appeared to correlate well with the disease status in bone marrow.		

Project Description:

The mechanism whereby *C. parvum* enhanced the survival of tumor-grafted AKR mice was investigated by *in vitro* cytolytic assay. In *c. parvum* treated mice, the peripheral blood monocytes were cytolytic for the syngeneic lymphoma cells. In contrast, untreated AKR mice have monocytes that are inactive against the lymphoma cells. The non-adherent lymphocytes from peripheral blood or spleen were not cytolytic against lymphoma cells, whether the mice were treated with *C. parvum* or not. Although untreated mice have natural cytolytic macrophages in the peritoneal cavity, their role in tumor resistance is limited. This is demonstrated by the observation that depletion of peritoneal macrophages with silica or carageenan could shorten the survival of AKR mice, only if the dose of i.p. injected lymphoma cells were 10^5 or less. The similar depletion of peritoneal macrophages with silica or carageenan did not abolish the effect of *c. parvum* in enhancing the survival of tumor transplanted mice.

These results suggest that the survival of mice is correlated with systemic monocyte activation which retarded the growth of metastatic tumor. This is also supported by the observation that subcutaneous injection of a mixture of peritoneal macrophage and lymphoma cells (ratio 100/1, 1000/1) did not lead to increased survival despite the fact that the growth of local tumor was strongly suppressed.

Despite the presence of cytolytic macrophages, AKR succumbed to widespread tumor when transplanted with as few as 100 lymphoma cells i.p. The existence of a subclone of AKR lymphoma cells that is resistant to macrophages was investigated. Three independently established AKR lymphoma cells were developed (A1, A2, A3). These lymphoma cells were virtually identical in morphology under the electron microscope. Their doubling time were also similar, 10.5 hours, yet their susceptibility to AKR macrophages were markedly different. A-1 cells were resistant and A-3 cells were easily lysed by macrophage. Further cloning is in progress and the biochemical basis for their different sensitivity to macrophages is being investigated.

In an attempt to study the host defense mechanism against human myeloid leukemia, we studied the natural killer cell activities against K-562, a human chronic myelocytic leukemia cell line and HL-60, a human acute promyelocytic leukemic cell line. The peripheral blood mononuclear cells, isolated from Ficoll-Hypaque gradient centrifugation was used as the effector cells. Four populations of individuals were studied: 1) Normal healthy adults (ages 19 to 70); 2) patients with acute nonlymphocytic leukemia remission treated with maintenance chemotherapy; 3) patients with acute nonlymphocytic leukemia receiving immunotherapy (allogeneic, neuraminidase treated myeloblasts) in addition to maintenance chemotherapy; and 4) patients with acute nonlymphocytic leukemia presented or relapsed in bone marrow without circulating blasts in their peripheral blood. We observed that patients with acute nonlymphocytic leukemia have significantly less natural killer cell activities against HL-60 (but not K-562), as compared to the normal control group. There was, however, no significant difference in natural killer cell activities between the groups that received chemotherapy only and chemo-immunotherapy. In patients presented or relapsed with acute nonlymphocytic leukemia, markedly diminished natural killer cell activities were observed. It is suggested that the immunotherapy with allogeneic neuraminidase treated blasts did not alter the natural killer cell activities in patients with acute nonlymphocytic leukemia.

SUMMARY REPORT

LABORATORY OF MOLECULAR BIOLOGY

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1979 - September 30, 1980

During the last year, the efforts of the Laboratory of Molecular Biology have focused on biochemical problems related to leukemia and other forms of cancer. For example, considering the roles that nucleases may play in disease, we became interested in interferon and the possibility that part of the biological activity of this protein may derive from a contaminating RNase. Purification of lymphoblastoid interferon by several steps, including affinity chromatography resulted in material free of measurable ribonuclease activity, indicating that exogenous RNase does not play a role in the mode of action of this interferon. This method of purification of IF may prove useful for isolating other RNase-free interferons since our results show that leukocyte IF, purified 300 fold by a different method than that used for lymphoblastoid IF, still demonstrated considerable RNase activity.

In other studies, an important effect of interferon on cells was elucidated. Highly purified mouse fibroblast interferon was found to induce a specific endonuclease in mouse Erlich ascites tumor cells. This enzyme requires ATP and double stranded RNA for activity and cleaves single stranded RNA and provides a molecular basis for a linkage between IF and RNA metabolism.

The production of IF by single mouse L939 cells was interesting in that, although every cell can be induced to produce interferon by Newcastle disease virus, the amount of IF varies from cell to cell. If poly(I) poly(C) was used for induction, the number of cells synthesizing IF was proportional to the amount of polynucleotide. However, each cell produces a quantity of IF that is not related to the quantity of double stranded polynucleotide inducer used.

We have worked closely with members of the Clinical Oncology Branch in a number of productive collaborations. These collaborations were fruitful because well documented patient material, in the form of serum, cells, or urine could be utilized in our studies. In certain cases, human samples were studied using original technology we developed whereby multiple enzyme activities could be visualized directly on polyacrylamide gels. Different classes of enzyme activity, such as RNases or DNases, are readily studied by including a polynucleotide substrate within the gel matrix. This method, polynucleotide-polyacrylamide gel electrophoresis, is fast, inexpensive and very sensitive. For example, only 10 picograms of bovine pancreatic RNase A are required to obtain a band of activity, while the use of supernatant from $2.5-5.0 \times 10^5$ sonicated human cells results in discrete enzyme activity banding patterns. If necessary, resolution of activities may be improved by using two-dimensional electrophoresis, with a substrate in the second dimension only. Results to date indicate the presence, in the granulocytes of patients with CML, of three DNases not found in individuals having AML or in normal volunteers. These activities are not related, as in a monomer-polymer relationship, to the enzymes of normal cells. The correlation of

enzyme presence with tumor load and treatment is underway, using serial samples obtained from patients prior to therapy, during therapy and after achievement of maximal response. Interestingly enough, in terms of animal models, an increase in the number and intensities of leukocyte DNases precedes the appearance of overt disease in AKR/J mice.

Such time course studies have proven useful in the examination of the multiple (22) RNase activities present in human serum. Considering an individual patient, distinct banding patterns are obtained prior to treatment, during treatment, and in remission. Patterns differ, however, from person to person and computer techniques are currently being used to determine if there is a band or combination of bands that will accurately predict a patients response to a course of treatment.

In addition to the above clinical studies, we are examining the causative agents of mouse leukemia at the molecular level in attempts to understand the functions of viral proteins. Since reverse transcriptase (RT) of Rauscher leukemia virus, purified free of the viral core (gag) proteins, is not capable of synthesizing infectious DNA, we studied the interaction of RT with these proteins on the assumption that in the intact virion the DNA synthesizing apparatus consists of RT and the gag proteins organized together in a specific manner. It was shown by us previously that one of these proteins (P30) and RT can form a complex with enhanced DNA polymerase activity. We now demonstrate that P12 can bind with RT and form two complexes; one has a molecular weight of 90,000 daltons and the other 127,000 daltons. P30 binds only with the 90,000 dalton material to form a RT-P12-P30 complex. This complex displays enhanced DNA synthesis and a marked improved fidelity of transcription compared to purified RT. We hypothesize that the 90,000 dalton RT-P12 complex may be a building block that, upon binding P30, forms a large DNA synthesizing apparatus.

Further support for such an assumption comes from studies of triton lysed virions. From these we have isolated two major complexes of molecular weights 600,000 and 200,000 daltons. Both of these complexes contain RT, P30 and P12. The heavier complex can disassociate to form the lower molecular weight complex which can further break-down to a P30 free RT-P12 complex. Functionally, the 600,000 dalton complex synthesizes DNA with much fewer errors than the lower molecular weight complexes or purified RT.

Besides aiding in our understanding of retroviral replication, studies of the complexes among viral core proteins and reverse transcriptase also are valuable in examining why certain retroviruses are oncogenic. We have detected complex formation in thymocytes and spleenocytes of AKR mice having a high incidence of spontaneous leukemia. The high molecular weight RT-core protein complex was first detected in thymus and spleen cells just prior to the onset of leukemia. The amount of complex then increased and was maximal when the leukemia was clinically manifested. Treatment of young animals with antiviral IgG, poly (A-U), or interferon delayed the appearance of both the complex and the development of leukemia. Characterization of the complex suggests that it may consist of ecotropic derived RT and xenotropic derived gag protein. If so, the oncogenic virus of AKR mice may be an ecotropic-xenotropic recombinant in the gag region.

In fact, we have isolated from AKR mice one such oncogenic virus. A cell-line was established from spontaneous lymphoma/leukemia cells of AKR mice though

alternating in vitro culturing with passage in mice. This cell line expresses a virus, which can infect, so far as known, only AKR thymocytes. Injection of these virally infected thymocytes into 2-4 week old AKR mice consistently produces tumors. Immunological characterization indicates that this new virus is a recombinant between ecotropic and xenotropic viruses: the envelope regions appear to originate from ecotropic virus whereas the xenotropic virus supplied the gag region.

Other work on viruses in progress centers on the fate of viral DNA sequences within different types of cells. For example, using hamster cells transformed by herpes simplex virus type 2 (HSV-2), a large DNA fragment of the viral genome was found within cell nuclei. Preliminary data suggests that the fragment is integrated into cellular DNA. In collaboration with members of the Clinical Oncology Branch, lymphoblastoid cell DNA is being analyzed for sequence homology with DNA from various herpesviruses. A DNA fragment common to HSV-1 and HSV-2 has been detected in certain lymphoblastoid cells. The presence of portions of the viral gene within these normal cells is exciting and we are pursuing studies to determine if viral specific proteins are expressed within the cells by using both in vitro and in vivo techniques.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)		U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 CM 06242-10 LMB	
PERIOD COVERED October 1, 1979 to September 30, 1980					
TITLE OF PROJECT (80 characters or less) Regulation of Poly(A) Induced RNase Inhibition by Metals or Polyamines					
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT					
PI:		T. P. Karpetsky		Sr. Investigator LMB, NCI	
Other		C.C. Levy		Chief LMB, NCI	
COOPERATING UNITS (if any) None					
LAB/BRANCH Laboratory of Molecular Biology					
SECTION					
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201					
TOTAL MANYEARS:		PROFESSIONAL:		OTHER:	
0.8		0.5		0.3	
CHECK APPROPRIATE BOX(ES)					
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER					
☐ (a1) MINORS ☐ (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords)					
Polynucleotide phosphorylase was used to covalently link poly(A) tracts, having 5,18,31,41, 59, 73, 100 and 150 residues to the 3' termini of <i>E. coli</i> [³H] rRNA (5S [³H] rRNA · (A)_n). Upon hydrolysis of these polyadenylated polynucleotides by ribonucleases from <i>Citrobacter</i> sp., <i>Enterobacter</i> sp., bovine pancreas, human spleen and human plasma, it was found that the (A)_n segment inhibited RNase activity, and that the inhibition was enhanced as the polyadenylic acid tract was lengthened. Certain polyamines, and polyamine analogs, restore enzyme activity in the presence of 5S rRNA · (A)_n. Changing the ionic strength caused fluctuations in the amount of enzyme inhibition, as did alterations in either reaction temperature or quantities of substrate and enzyme. In all systems studied, inhibition of RNase activity mediated by 5S rRNA · (A)_n may be closely regulated by the chain length of the poly(A) segment and the concentrations of the components of the reaction mixture noted above. Furthermore, use of poly(A) covalently linked to substrate, as 5S rRNA · (A)_n, frequently resulted in greater inhibitions than if equal quantities of free poly(A) were used. With several enzymes, in the absence of inhibition, the substrate portion of 5S rRNA · (A)_n was hydrolyzed more rapidly than the same amount of 5S rRNA, implying that the structure of 5S rRNA is altered upon covalent attachment of poly(A).					

The synthesis of poly(A) tracts having 5, 18, 31, 41, 59, 73, 100 and 150 residues, covalently attached to the 3' termini of *E. coli* 5S [³H] rRNA (5S rRNA-(A)_n) was carried out with polynucleotide phosphorylase. These polyadenylated RNAs and unmodified 5S rRNA were utilized as substrates for ribonucleases from *Citrobacter*, *Enterobacter*, bovine pancreas, human spleen and human plasma. In all cases, short lengths of poly(A) (n=18) induce a powerful inhibition (38-87%) of RNase activity, which increases as the poly(A) tract is lengthened. The poly(A) mediated protection of substrate from degradation extends to RNA molecules which are not covalently linked to poly(A), demonstrating that a sub-population of poly(A) containing RNA molecules can contribute to the stability of other RNAs by inhibiting the RNase mediated hydrolysis of these substrates. The structure of 5S rRNA (T_m=61°C) is altered upon covalent attachment of poly(A) (T_m=46°C if 31 adenylic acid residues are present at the 3' termini). Thus, under certain conditions, 5S rRNA · (A)_n behaves quite differently from a mixture of 5S rRNA and poly(A). Using RNases from either bovine pancreas or human spleen, it was found that covalent attachment of (A)_n to the substrate molecule (5S [³H] rRNA) produced an inhibitor superior to free (A)_n. The ratio of K's ((A)_n attached/(A)_n free), for example, was 0.5 using the human spleen RNase. Additionally, under certain circumstances, the substrate portion of 5S [³H] rRNA · (A)_n behaves quite differently from 5S [³H] rRNA as the following example demonstrates. Increasing the ionic strength of a mixture containing human plasma RNase and either 5S [³H] rRNA · (A)_n or 5S [³H] rRNA+(A)_n induces a reversal of inhibition in either case. Enzyme activity for 5S [³H] rRNA+(A)_n never exceeds that for 5S [³H] rRNA alone, i.e., the fractional activity is always < 1.0, regardless of the ionic strength. However, using 5S [³H] rRNA · (A)_n, fractional activities of > 1.5 are obtained at moderate ionic strengths. These results suggest that in the absence of inhibition by (A)_n, the substrate portion of 5S [³H] rRNA · (A)_n is more susceptible to hydrolysis by this enzyme than is 5S [³H] rRNA alone. Furthermore, polyadenylated substrate and unpolyadenylated substrate behaved differently in reaction mixtures containing RNase A and any of a variety of divalent cations. Comparing RNase reaction mixtures that contain either 5S [³H] rRNA and metal ion or 5S [³H] rRNA · (A)_n and metal ion, the maximum activity for the latter case was 40% more than that of the former. This would appear to result from the structural destabilization produced in 5S [³H] rRNA concomitant with covalent addition of 3'-terminal (A)_n segments. Also the dependence of RNase inhibition upon temperature may be quite different, depending upon whether poly(A) is covalently linked to substrate or not. For example, in the presence of free poly(A), the inhibition of human spleen RNase undergoes a dramatic reversal between 30° and 37°, such that there is a virtually complete restoration of enzyme activity, above the latter temperature. The inhibition affected by the poly(A) segments of 5S [³H] rRNA · (A)_n, however, is essentially independent of temperature between 20° and 37°C. Higher temperatures result only in partial restoration of enzyme activity (75% at 61°C). Thus, although poly(A) covalently linked to 5S [³H] rRNA and free poly(A) induce similar inhibitions of human spleen RNase activity below 37°, at this temperature or above it, the bound polynucleotide is the superior inhibitor.

The reversal of poly(A) induced inhibition may be achieved in a number of ways. Regulatory effects may be combined in such a way that, for fixed polyadenylated substrate and enzyme concentrations, a wide variety of RNase activities and inhibitions may be achieved. The addition of both spermidine and magnesium to

individual reaction mixtures containing inhibited enzyme provides one such example. For fixed magnesium concentrations ranging from 0-50 μM , as the spermidine concentration is increased from 0 to 20 μM , 3'-terminal poly(A) segment inhibition of RNase activity is largely overcome and the rate of substrate processing increased substantially. Also, if constant quantities of spermidine are present, raising the quantity of metal ions restores RNase activity. The concentrations and identities of ions in reaction mixtures exert a profound influence on enzyme inhibition. Factors such as substrate and enzyme coconcentrations and temperatures of incubation can regulate RNase activity as well. Poly(A) induced inhibition of RNase activity is not a simple process, but one subject to considerable control by factors in the environment of the enzyme and inhibitor. In certain cases, the amount of inhibition also depends upon whether or not the inhibitor is covalently linked to substrate.

Publications:

Karpetsky, T.P., Shriver, K., and Levy, C.C.: Covalent linkage of poly(A) to RNA produces a molecule altered in both structure and susceptibility to RNase mediated hydrolysis. *J. Biol. Chem.* 255: 2713-2721, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06933-05 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Ribonuclease Activities in Human Sera		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	T. Karpetsky	Sr. Investigator
		LMB, NCI
Other:	C. Levy P. Wiernik	Chief Chief
		LMB, NCI CO, NCI
COOPERATING UNITS (if any) Clinical Oncology Branch, BCRP, DCT, NCI		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.3	OTHER: 0.3
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS		
☒ (b) HUMAN TISSUES		
☐ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The members of seventeen families, comprising seventy-nine normal volunteers, having no clinical evidence of disease, were examined for multiple <u>ribonuclease</u> activities using <u>polynucleotide-polyacrylamide gel electrophoresis</u> . Gels (6.5%) were prepared containing <u>polyadenylic acid (.05%)</u> . Serum samples (15 μ l) were electrophoresed using conditions under which enzyme catalyzed hydrolysis of the polynucleotide did not occur (pH 8.6). After electrophoresis, gels were incubated and stained, revealing RNase activities as colorless bands in otherwise purple gels. The high resolution obtained with this technique enables 22 distinct activity bands to be visualized. Although no two individuals had identical activity patterns, a statistical profile of a normal serum pattern was obtained. These studies are the basis for examination of activity patterns of individuals having leukemia. Serum RNase patterns of patients change in response to treatment of disease. Coincident with treatment, certain enzymes appear or disappear, and the intensities of some bands undergo marked alterations.		

In order to understand how changes in serum ribonuclease activity banding patterns may be related to the tumor load or clinical status of leukemia patients, these patterns were first examined using 79 normal volunteers consisting of the members of 17 families (34 parents, 45 children). Polynucleotide-polyacrylamide gel electrophoresis was used to obtain activity patterns. Acidic (negatively charged) RNases from human serum were resolved and visualized using 6.64% polyacrylamide gels containing 0.05% polyadenylic acid (poly(A)). Gels were prepared using Tris-HCl buffer, pH 8.9, and had a 3.02% polyacrylamide stacking gel containing no polynucleotide. After electrophoresis of 15 μ l of serum (pH 8.6, 6 mA/gel, 4°C), gels were incubated in 7% acetic acid at room temperature. Staining (0.1% Pyronin Y overnight) and destaining resulted in reddish-purple gels having clear bands indicating regions of enzyme activity. Gel patterns were found to be reproducible, i.e., a sample run many times gave the same activity bands. Standard deviations of individual band positions are small. Distinct patterns were obtained from different individuals. No two people had identical patterns, thus increasing the complexity of interpretation of changes in banding pattern. Using an algorithm, however, 22 windows (or upper and lower boundaries in terms of R_f) for the presence of individual gel bands may be defined. No individual has less than 7 or more than 18 bands. Children have a somewhat different average gel pattern than that of parents. Certain bands (# 7, 14, 15 and 20) are found more often in parents, compared to children, while other bands (# 18) occur more frequently in children than parents. Genetically related family members have less differences among their serum banding patterns than unrelated individuals. Although the presence of a band does not absolutely define the presence or absence of any other band, statistically significant links between certain bands are found.

With this information in hand, leukemia patients are currently being examined during the course of their treatment for changes in serum RNase banding patterns. Although firm conclusions will have to await adequate patient sampling, preliminary results indicate that large changes in pattern, as well as in certain individual band intensities, occur in response to treatment. The possible predictive value of the changes regarding the patients' clinical course is currently under investigation.

Publications:

Levy, C.C. and Karpetsky, T.P.: Human Ribonucleases. In Holcenberg, J. (Ed): Enzymes as Drugs. New York, Wiley and Sons, 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06980-03 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Study of the Physiological Importance of Reverse Transcriptase-P30 Interaction		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	A. Bandyopadhyay	Sr. Investigator LMB, NCI
Other:	M. Purtell C. Levy K. Chang R. Friedman	Clinical Associate Chief Chief Chief LMB, NCI LMB, NCI LCBGY, NCI LEP, NIAID
COOPERATING UNITS (if any) Dr. Donald C. Fish, Frederick Cancer Research Center; Dr. J. Bookout, Microbiological Associates; J. Eichberg, Southwest Foundation for Research and Education, San Antonio, Texas 78284; Dr. Stanford Moore and Dr. D. Wang, Rockefeller University		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: .5
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Reverse transcriptase</u> (RT) and p30 from several <u>retroviruses</u> have been shown to bind together specifically to form a high molecular weight complex. This complex displays increased <u>DNA-polymerase</u> activity over that shown by RT alone. Such a complex was detected in the <u>thymocytes</u> and <u>spleenocytes</u> of <u>AKR mice</u> prior to the onset of disease. The amount of the complex is maximum when the clinical symptoms manifest. The complex can be seen only in the target organs. In the animals treated with either antiviral <u>IgG</u> or <u>poly(A-U)</u> or <u>interferon</u> which delayed the development of spontaneous <u>leukemia</u> , the complex appeared much later in life but prior to the development of disease. Further characterization of the complex suggests that p30 of the complex is more related to <u>xenotropic virus</u> .		

Since both p30 and reverse transcriptase are present in the virion core and p30 stimulates DNA synthesis, it was postulated that they remain in an organized molecular structure and efficiently synthesize DNA. This coupled with the actual isolation of reverse transcriptase-p30 complex from the virion itself suggested to us that the formation of the complex may be a pre-requisite of the assembly of the virus or for activating the oncogenicity of the virus. In this connection, a reverse transcriptase-p30 complex was isolated from the sub-cellular fractions of thymocytes and spleenocytes of AKR/J mice (which develop spontaneous leukemia at 28 weeks of age) starting at 15 to 20 weeks of age. Such complex formation is maximal when the clinical symptoms become manifest. Interestingly, the complex is presently only in the target organs, e.g., thymus and spleen whereas either non-target organs are devoid of such complex. However, the animals treated with antiviral IgG or poly(A-U) or interferon which delayed the development of spontaneous leukemia, the complex formation appear much later in life, but prior to the development of disease.

Since AKR thymocytes can express both ecotropic and xenotropic virus prior to the development of leukemia, the role of these viruses towards the formation of reverse transcriptase-p30 complex was further investigated. Reverse transcriptase and p30 was purified from ecotropic (AKR type) virus, xenotropic virus and from the thymic lymphoma cells. When immune technique was applied to relate the proteins, results clearly suggested that the p30, but not the reverse transcriptase, from lymphoma cells differed from those of ecotropic virus and was similar to xenotropic virus. Further support that p30 is more related to xenotropic virus comes from the salt-dissolution studies of the complex of the thymic lymphoma cells.

Publications:

Bandyopadhyay, A.K., Chang, E.H., Levy, C.C. and Friedman, P.: Structural abnormalities of murine leukemia virus produced by interferon treated cells. *Biochem. Biophys. Res. Comm.* 87: 983-988, 1979.

Eichberg, J., Kalter, S.S., Heberling, R.L., Lawlor, D.A., Morrison, J.D., Bandyopadhyay, A.K. and Levy, C.C.: In vivo activation of endogenous virus. *Nature*, in press, 1980.

Bandyopadhyay, A.K., Wang, D. and Levy, C.C.: Complexing Rauscher leukemia virus reverse transcriptase with polyspermine RNase. *Biochem. J.*, in press, 1980.

Bandyopadhyay, A.K., Fish, D.C. and Levy, C.C.: Existence of reverse transcriptase p30 complex in AKR mice after a high incidence of spontaneous lymphocytic leukemia. *J. Gen. Virol.* in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06982-03														
PERIOD COVERED October 1, 1979 to September 30, 1980																
TITLE OF PROJECT (80 characters or less) Binding Between Reverse Transcriptase and Hodgkin's Disease Patients' RNase																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">A. Bandyopadhyay</td> <td style="width: 30%;">Sr. Investigator</td> <td style="width: 20%;">LMB, NCI</td> </tr> <tr> <td rowspan="3">Other:</td> <td>C. Levy</td> <td>Chief</td> <td>LMB, NCI</td> </tr> <tr> <td>P. Wiernik</td> <td>Chief</td> <td>CO, NCI</td> </tr> <tr> <td>C. Diggs</td> <td>Sr. Investigator</td> <td>CO, NCI</td> </tr> </table>			PI:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI	Other:	C. Levy	Chief	LMB, NCI	P. Wiernik	Chief	CO, NCI	C. Diggs	Sr. Investigator	CO, NCI
PI:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI													
Other:	C. Levy	Chief	LMB, NCI													
	P. Wiernik	Chief	CO, NCI													
	C. Diggs	Sr. Investigator	CO, NCI													
COOPERATING UNITS (if any) Clinical Oncology Branch, BCRP, DCT, NCI																
LAB/BRANCH Laboratory of Molecular Biology																
SECTION																
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201																
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1														
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																
SUMMARY OF WORK (200 words or less - underline keywords) It was shown previously that <u>Rauscher leukemia virus reverse transcriptase</u> can specifically bind with plasma RNase of <u>Hodgkin's disease</u> patients. (J. Biol. Chem. 252, 7783-7787, 1977). Extension of this study to other viral reverse transcriptase indicates that the plasma RNase of Hodgkin's disease patients is unable to bind with reverse transcriptase purified from either endogeneous, mouse tropic viruses (e.g., AKR and NZB) or Feline leukemia virus or Simian Sarcoma Virus. In contrast, this plasma RNase can easily complex with the reverse transcriptase purified from either Moloney or Friend leukemia virus. The binding of reverse transcriptase to plasma RNase obtained at various stages of Hodgkins disease are now being evaluated.																

It was demonstrated in this laboratory that Rauscher leukemia virus reverse transcriptase can specifically bind with the plasma RNase of Hodgkin's disease patients. This work was extended to examine the specificity of the binding ability of the plasma RNase of Hodgkin's disease patients to reverse transcriptases of other retroviruses.

Reverse transcriptases were purified by sequential column chromatography from several retroviruses. These DNA synthesizing enzymes were exposed to Hodgkin's patient plasma RNase to examine the complexing ability. Results indicated that the patient's plasma RNase was unable to bind with reverse transcriptase purified from either endogenous mouse tropic virus e.g., AKR-ecotropic, NZB-xenotropic, or cat tropic virus. Even two primate virus reverse transcriptases, e.g. Simian sarcoma virus and baboon endogenous virus, were not capable of complexing with patient plasma RNase. In contrast, reverse transcriptase of either Moloney leukemia virus or Friend leukemia virus can complex with plasma RNase.

Studies of the binding between reverse transcriptase purified from human leukemic cells and plasma RNase of Hodgkin's disease patients are now being conducted. Furthermore, the complexing ability of Rauscher leukemia virus reverse transcriptase and RNase of serum obtained at various stages of disease are now being evaluated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06984-03 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Reverse Transcriptase and Viral Protein Interaction in Relation to DNA Synthesis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	A. Bandyopadhyay	Sr. Investigator
Other:	T. Karpetsky C.C. Levy M. Purtell K. Chang R. Gallagher	Sr. Investigator Chief Clinical Associate Chief Sr. Investigator
		LMB, NCI LMB, NCI LMB, NCI LCBGY, NCI LMB, NCI
COOPERATING UNITS (if any) Dr. Ralph Arlinghaus, University of Texas; Dr. P. Pitha, Interferon Lab. Johns Hopkins University Cancer Center		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.6	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) It was shown previously that two core proteins of Rauscher leukemia virus, reverse transcriptase (RT) and p30 interact with each other and form a complex with enhanced DNA synthesis. When, however, RT was interacted with another core protein p12, two molecular size of complexes can be seen: one with high molecular weight and another low molecular weight. High molecular weight RT-p12 does not interact with p30. In contrast, low molecular RT-p12 interacts with p30 and forms a large molecular weight complex RT-p12-p30. Such a complex is capable of increasing the synthesis of DNA with a greater fidelity than RT alone. We hypothesize that low molecular weight RT-p12 may be a building block that, upon binding p30, forms a large <u>DNA synthesizing apparatus</u> .		

We have previously shown that Rauscher leukemia virus reverse transcriptase can specifically bind with its own major internal protein p30 and form a large molecular aggregate. Such a complex enhances DNA synthesis.

We now demonstrate that another core protein, p12, can also bind with reverse transcriptase and stimulate DNA synthesis several fold over reverse transcriptase alone. Such complex formation can take place at 1 mM Mn^{++} , 0.12 M KCl and pH 6.5 to 8.5. When RT-p12 mixtures were subjected to glycerol gradient centrifugation, it revealed two kinds of RT-p12 complex: one has a molecular weight of 170,000 and the other one 90,000. These two complexes differ on their binding ability with p30. The high molecular weight RT-p12 complex is unable to bind with p30. In contrast, 90,000 mw RT-p12 can easily form a large molecular weight (molecular weight greater than 300,000) complex of RT-p12-p30. This complex of RT-p12-p30 can synthesize DNA several times faster than the reverse transcriptase alone.

When DNA, synthesized by the individual complexes, was compared, the best fidelity was seen in DNA synthesized by RT-p12-p30 complex. Highest infidelity was recorded in DNA synthesized by reverse transcriptase alone. RT-p30 and RT-p12 have better fidelity in DNA synthesis than RT alone, but not nearly as great as RT-p30-p12 complex.

We hypothesize that the 90,000 daltons complex may be a building block that, upon binding p30, forms a large DNA synthesizing apparatus. The significance of the 170,000 dalton complex is unclear, but its inability to bind p30 suggests that RT-p12 interaction in this complex differs from that in 90,000 dalton complex. The functional properties and the degree of p12 phosphorylation of these complexes is under investigation.

Publications:

Purtell, M., Meric, A., Bandyopadhyay, A.K. and Levy, C.C.: Multiprotein complexes of Rauscher leukemia virus. *J. Biol. Chem.*, in press, 1980.

Bandyopadhyay, A.K. and Levy, C.C.: Type specific interaction between reverse transcriptase and p30 in mouse tropic virus. *J. Gen. Virol.*, in press, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06986-03 LMB								
PERIOD COVERED October 1, 1979 to September 30, 1980										
TITLE OF PROJECT (80 characters or less) Control Mechanisms by RNase C Type Virus Replication in the Balb/C Mouse										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">A. Bandyopadhyay</td> <td style="width: 25%;">Sr. Investigator</td> <td style="width: 20%;">LMB, NCI</td> </tr> <tr> <td>Other:</td> <td>C. Levy</td> <td>Chief</td> <td>LMB, NCI</td> </tr> </table>			PI:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI	Other:	C. Levy	Chief	LMB, NCI
PI:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI							
Other:	C. Levy	Chief	LMB, NCI							
COOPERATING UNITS (if any) Dr. Jack Bookout, Microbiological Associates										
LAB/BRANCH Laboratory of Molecular Biology										
SECTION										
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201										
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3								
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">☐ (a) HUMAN SUBJECTS</td> <td style="width: 33%;">☐ (b) HUMAN TISSUES</td> <td style="width: 33%;">☒ (c) NEITHER</td> </tr> <tr> <td>☐ (a1) MINORS</td> <td colspan="2">☐ (a2) INTERVIEWS</td> </tr> </table>			☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER	☐ (a1) MINORS	☐ (a2) INTERVIEWS			
☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER								
☐ (a1) MINORS	☐ (a2) INTERVIEWS									
SUMMARY OF WORK (200 words or less - underline keywords) <u>In vitro</u> and <u>in vivo</u> experimentation has shown that infection with <u>murine sarcoma virus</u> (Mo-MSV (MLV)) resulted in rapid decreases in amounts of <u>cytoplasmic ribonuclease activity</u> which correlated inversely with levels of viral specific cytoplasmic <u>mRNA</u> and with concentrations of <u>reverse transcriptase</u> and <u>p30</u> associated with the microsomal fractions. Similar changes occurred when mice were infected with <u>murine leukemia virus</u> (Mo-MLV) at later periods when leukemia began to be observed. Injections of Poly A:U or interferon <u>suppressed virus expression</u> in spleen lymphocytes.										

Spleen cells from mice injected with MSV (MLV) showed elevations in levels of p30, reverse transcriptase, and virus specific mRNA which peaked in the cell cytoplasm during the time of maximum fibrosarcoma growth and decreased as the tumors spontaneously regressed. During the period of maximum viral expression in the spleen cells the levels of RNase associated with the polysomes dropped off drastically, then rose again during regression. Maximum viral expression with decreased RNase levels were also observed during the later stages of infection with MLV alone. These observations suggest that some form of synergism exists between the early expression of C type virus in spleen lymphocytes and tumor expression in the animal. Since multiple poly A:U or interferon treatments suppress the accumulation of viral mRNA within the cytoplasm of spleen lymphocytes, a regulatory role of a polysomal RNase is postulated.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06994-02 LMB																								
PERIOD COVERED October 1, 1979 to September 30, 1980																										
TITLE OF PROJECT (80 characters or less) Characterization of an oncogenic virus isolated from AKR lymphoma cells																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																										
<table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 35%;">W. Williams</td> <td style="width: 35%;">Clinical Associate</td> <td style="width: 20%;">LMB, NCI</td> </tr> <tr> <td rowspan="5">Other:</td> <td>A. Bandyopadhyay</td> <td>Sr. Investigator</td> <td>LMB, NCI</td> </tr> <tr> <td>M. Purtell</td> <td>Clinical Associate</td> <td>LMB, NCI</td> </tr> <tr> <td>C.C. Levy</td> <td>Chief</td> <td>LMB, NCI</td> </tr> <tr> <td>L. Shu</td> <td>Clinical Associate</td> <td>LCB, NCI</td> </tr> <tr> <td>J. Sutherland</td> <td>Assoc. Professor</td> <td>CO, NCI</td> </tr> <tr> <td></td> <td>R. Gallagher</td> <td>Sr. Investigator</td> <td>LMB, NCI</td> </tr> </table>			PI:	W. Williams	Clinical Associate	LMB, NCI	Other:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI	M. Purtell	Clinical Associate	LMB, NCI	C.C. Levy	Chief	LMB, NCI	L. Shu	Clinical Associate	LCB, NCI	J. Sutherland	Assoc. Professor	CO, NCI		R. Gallagher	Sr. Investigator	LMB, NCI
PI:	W. Williams	Clinical Associate	LMB, NCI																							
Other:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI																							
	M. Purtell	Clinical Associate	LMB, NCI																							
	C.C. Levy	Chief	LMB, NCI																							
	L. Shu	Clinical Associate	LCB, NCI																							
	J. Sutherland	Assoc. Professor	CO, NCI																							
	R. Gallagher	Sr. Investigator	LMB, NCI																							
COOPERATING UNITS (if any) Dr. J. Bookout, Microbiological Associates; Dr. Robert Neuman, Dental School, University of Maryland																										
LAB/BRANCH Laboratory of Molecular Biology																										
SECTION																										
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201																										
TOTAL MANYEARS: 0.5	PROFESSIONAL: .4	OTHER: .1																								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																										
SUMMARY OF WORK (200 words or less - underline keywords) A <u>cell line</u> was established from spontaneous <u>lymphoma/leukemia</u> of an <u>AKR mouse</u> through alternating <u>in vitro</u> culturing with passage in mice. After the fourth successive <u>in vitro/in vivo</u> passage, the virus associated with this line was altered in several properties from that of the original. Recent virus populations appear to be <u>defective virus-helper virus</u> mixtures with the potential to <u>transform thymocytes in vitro</u> .																										

An AKR/J lymphoma cell line (line C) was established from a spontaneous lymphocytic lymphoma/leukemia by alternating *in vitro* culturing with passage in 8-12 week old AKR mice. Cells grew in animals into defined tumors containing lymphoid cells. After the third cycle of *in vitro/in vivo* passage, line C was partially characterized. Though virus particles with high reverse transcriptase activity were released from the cells, levels of structural components (p30, p15) were difficult to detect within the cell. Standard assays for infectivity of the particles (mink plaque, assay, XC plaque assay, focus assay on mink $S^+ L^-$ cells) were negative.

After the 4th *in vivo* passage line C showed improved growth rates when cultured *in vitro*. Direct assay of 4th passage line C cells on XC cultures was positive and plaques on XC monolayers were made with NIH-3T3 cells but not with mink cells after infection of these cells with 4th passage virus supernatant. Further, 4th passage virus transformed thymocytes *in vitro*, transmitting to them the ability to produce lethal tumor masses composed of lymphoid cells in young adult mice by 2 weeks post inoculation. Uninfected thymocytes both normal and PHA-stimulated failed to induce tumors while PHA-stimulated thymocytes infected with virus were oncogenic but with a slower rate of tumor development. These preliminary results suggest that the 4th passage virus may be a defective virus-helper virus mixture with the potential to transform lymphoid cells *in vitro*. Further experimentation is planned to confirm these results and to explore the biological capabilities of this virus.

Neonatal thymocytes exposed to filtered tissue culture supernatant of this cell line produced lymphomas within 17-30 days of subcutaneous injection into syngeneic mice. Neonatal thymocytes unexposed to the tissue culture supernatant or thymocytes that were infected with AKR MCF (Mink Cell Focus Forming) virus failed to produce tumors within this time interval. Cloned and uncloned tumor cells produced C-type particles (by E.M criteria) with high reverse transcriptase activity and 70S RNA. Preliminary examination of these particles by immunological techniques indicated that the core proteins may be a mixture of N-tropic and Xenotropic proteins whereas the envelope protein GP70 may be N-tropic and unrelated to MCFGP70. These studies suggest that the viral complex may be a new oncogenic virus distinct from previous AKR isolates and that it may have arisen from a recombination in the gag region of the genome of N-tropic and Xenotropic viruses.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09101-02 LMB																
PERIOD COVERED October 1, 1979 to September 30, 1980																		
TITLE OF PROJECT (80 characters or less) Characterization of DNases in Normal and Leukemic Leukocytes																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">G. Brown</td> <td style="width: 35%;">Staff Fellow</td> <td style="width: 15%;">LMB, NCI</td> </tr> <tr> <td>Other:</td> <td>T. Karpetsky</td> <td>Sr. Investigator</td> <td>LMB, NCI</td> </tr> <tr> <td></td> <td>C.C. Levy</td> <td>Chief</td> <td>LMB, NCI</td> </tr> <tr> <td></td> <td>P. Wiernik</td> <td>Chief</td> <td>CO, NCI</td> </tr> </table>			PI:	G. Brown	Staff Fellow	LMB, NCI	Other:	T. Karpetsky	Sr. Investigator	LMB, NCI		C.C. Levy	Chief	LMB, NCI		P. Wiernik	Chief	CO, NCI
PI:	G. Brown	Staff Fellow	LMB, NCI															
Other:	T. Karpetsky	Sr. Investigator	LMB, NCI															
	C.C. Levy	Chief	LMB, NCI															
	P. Wiernik	Chief	CO, NCI															
COOPERATING UNITS (if any) Clinical Oncology Branch, BCRP																		
LAB/BRANCH Laboratory of Molecular Biology																		
SECTION																		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201																		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.25	OTHER: 0.25																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Polyacrylamide gels</u> containing DNA are used to separate and visualize individual acidic <u>DNase</u> activities derived from peripheral <u>leukocyte</u> subpopulations. Electrophoretic conditions are such that no destruction of substrate occurs during migration. After electrophoresis, incubation and staining reveals the presence of <u>DNases</u>. Cell populations from normal human donors, which are highly enriched for <u>lymphocytes</u> exhibit enzyme activity profiles different from these obtained from populations highly enriched for <u>polymorphonuclear neutrophils</u>. These profiles show only slight variation among donors. Sharp pH optima, which vary with the identity of the incubation buffer, are exhibited. Proper ionic strength is critical in the development of activity during incubation. Satisfactory visualization of activities is obtained using only 10^6 cells/gel. Cell populations from human donors with leukemia often exhibit activity spectra different from normal donor spectra. These variant spectra appear to be characteristic of particular types of <u>leukemia</u>. Visualization of positively charged DNases is obtained by electrophoresis in one dimension lacking polynucleotide, followed by migration in a second dimension containing DNA. Mobility in the second dimension is made possible by the presence of polycations. The two-dimensional activity patterns appear to correlate with the course of treatment administered to individual patients.																		

The DNases within any mammalian cell have been very poorly characterized. However, it has been reported that there is a nuclease present in normal lymphocytes that is not present in leukemic lymphocytes.

This study is designed to investigate the nucleases present in leukocytes from normal individuals. These nucleases will be compared with those present in leukocytes from a variety of leukemias to determine whether these enzymes can serve as a disease marker.

We have chosen leukocytes as a study tissue because they can be easily obtained, highly purified in large amounts, and easily fractionated into subpopulations. We have prepared extracts from various cellular fractions. We are using these extracts and one- and two-dimensional activity gels (involving a wide variety of substrates) to determine a) the number of DNases present; b) activity and substrate specificity of the individual nucleases; c) cellular localizations; d) cofactors or modifiers involved and; e) whether any alteration in the DNases occurs as a consequence of leukemia and consequent treatment.

The assay system has demonstrated the following advantages. It is extremely sensitive, requiring only 10^6 cells/assay. It is very simple to use and requires only a minimum of sample manipulation, thus eliminating loss of specific activities. There is a high degree of resolution, i.e., as many as 20 individual activities have been resolved. Patterns from normal cells are confined to specific gel regions. Normal patterns and patterns associated with certain leukemias are very different and are readily scannable with a densitometer. The patterns are highly reproducible.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09125-01 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Interferon Mechanism of Action		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	G. Brown	Staff Fellow LMB, NCI
Other:	C.C. Levy	Chief LMB, NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<u>Interferon</u> has been shown to alter <u>RNA metabolism</u> by the induction of at least one <u>endonuclease</u>. Large changes in overall <u>RNase</u> activity are not normally induced by <u>interferon</u>. To extend these initial observations, we are looking at <u>RNase</u> and <u>DNase</u> activities in extracts derived from untreated and interferon treated cells using <u>polynucleotide polyacrylamide activity gel electrophoresis</u>. This system allows the detection and quantitation of individual activities rather than gross activity.		

Interferon inhibits both RNA and DNA virus replication. At least part of the inhibition is due to the induction of an endonuclease which requires ATP and double-stranded RNA for activation and which uses single-stranded RNA as the substrate. We are extending these initial observations. Our polynucleotide polyacrylamide activity gel assay system allows the easy detection and quantitation of individual nuclease activities and requires small amounts of starting materials.

We are preparing extracts from cells exposed to varying amounts of interferon for varying times. Aliquots of these extracts (from 10^6 cells per gel) are electrophoresed into the gels either directly or following incubation with potential nuclease activating or inactivating agents. Each extract is put onto a number of gels containing a variety of substrates. Following incubation and staining, the gels are scanned with a dual beam microdensitometer to quantitate nuclease expression.

Publications:

Brown, G.E., Simon, E.H. and Chung, C. Interferon Production by individual L cells. J. Gen. Virol. 47: 171-182, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09126-01 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Antiviral Activity of Some Oligo 5-Iodo-2'-deoxy-uridines		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	B. Ho	Sr. Investigator LMB, NCI
Other:	C.C. Levy	Chief LMB, NCI
COOPERATING UNITS (if any) Dr. Yung-Chi Cheng, Dept. of Pharmacology, University of North Carolina, Chapel Hill		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.9	OTHER: 0.1
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<u>Oligonucleotides of 5'-iodo-2'-deoxy-uridine</u> are synthesized by the triester method, i.e., all the intermediates plus starting nucleotide functional groups, such as 5', 3' hydroxyl groups and phosphate groups were protected. The 5' hydroxyl was protected with trityl groups and 3' hydroxyl by benzoyl groups. The phosphate was protected with <i>p</i>-Cl-phenol and β-cyanoethanol. Removal of protecting group was achieved by the treatment of 2% benzene sulfonyl tetrazole followed with the treatment of concentrated ammonia at 50°C. The effectiveness of oligo nucleotides thus synthesized are being tested against <u>Herpes simplex virus</u>.		

Oligonucleotides have been chemically synthesized since late 1960's. The purification involved in each step is tedious since ion exchange columns have to be employed. The triester method, which was developed recently by Narang et al., seemed to be quite promising. The purification involves mostly silica gel column chromatography or recrystallization. In our approach toward the synthesis of oligo 5-iodo-2'-deoxy-uridine, the 5'-hydroxyl group which does not involve in the coupling reaction, was protected with p -Cl-phenol and β -cyanoethanol. Thus, an anhydrous pyridine solution containing 5'- o -dimethoxytrityl-5-iodo-2'-deoxy-uridine 3'- o -chlorophenyl phosphate plus another 5-iodo-2'-deoxy-uridine containing a free 5'-hydroxyl and 3'- o -benzoyl group was coupled with trisopropyl benzene sulfonyl tetrazole. The product of fully protected dinucleotide was purified either on TLC plates or columns (silica gel). Removal of protecting groups was achieved by first treating the fully protected oligonucleotide with 2% Benzene sulfonic acid in chloroform-methanol solution (7/3) followed by treatment with concentrated ammonia at 50°C for 4-6 hours. The product of fully deprotected dinucleotide was purified on polyethylenimine PEI/UV₂₅₄ TLC plates.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09128-01 LMB																				
PERIOD COVERED October 1, 1979 to September 30, 1980																						
TITLE OF PROJECT (80 characters or less) The Synthesis of Infectious RLV DNA From Purified Viral Components																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">A. Bandyopadhyay</td> <td style="width: 35%;">Sr. Investigator</td> <td style="width: 15%;">LMB, NCI</td> </tr> <tr> <td rowspan="5">Other:</td> <td>M. Purtell</td> <td>Clinical Associate</td> <td>LMB, NCI</td> </tr> <tr> <td>A. Meric</td> <td>Jr. Investigator</td> <td>LMB, NCI</td> </tr> <tr> <td>S. Sargent</td> <td>Jr. Investigator</td> <td>LMB, NCI</td> </tr> <tr> <td>A. Andriuk</td> <td>Jr. Investigator</td> <td>LMB, NCI</td> </tr> <tr> <td>C. C. Levy</td> <td>Chief</td> <td>LMB, NCI</td> </tr> </table>			PI:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI	Other:	M. Purtell	Clinical Associate	LMB, NCI	A. Meric	Jr. Investigator	LMB, NCI	S. Sargent	Jr. Investigator	LMB, NCI	A. Andriuk	Jr. Investigator	LMB, NCI	C. C. Levy	Chief	LMB, NCI
PI:	A. Bandyopadhyay	Sr. Investigator	LMB, NCI																			
Other:	M. Purtell	Clinical Associate	LMB, NCI																			
	A. Meric	Jr. Investigator	LMB, NCI																			
	S. Sargent	Jr. Investigator	LMB, NCI																			
	A. Andriuk	Jr. Investigator	LMB, NCI																			
	C. C. Levy	Chief	LMB, NCI																			
COOPERATING UNITS (if any) None																						
LAB/BRANCH Laboratory of Molecular Biology																						
SECTION																						
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201																						
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0																				
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords) Although in the mammalian virion <u>reverse transcriptase (RT)</u> can replicate the genome, after purification from the viral core or <u>gag proteins</u> it loses this ability. This suggests that the gag proteins are important in insuring proper functioning of RT. Therefore, we pursued the hypothesis that in the intact virion there exists a <u>DNA synthesizing apparatus</u> consisting of RT and gag proteins organized in a specific manner. We have succeeded in uncovering certain gag protein-RT interactions utilizing both purified proteins and virion cores from <u>Rauscher leukemia virus (RLV)</u> and hope that soon for the first time we may be able to synthesize infectious DNA from highly purified viral components.																						

RT and P30 can associate to form a high molecular weight complex of approximately 400,000 daltons. This complex displays an increased DNA polymerase activity over RT alone. P12 also stimulates RT activity, and the stimulation is additive with P30. P12 binds with RT to form RT-P12 complexes of 170,000 and 90,000 daltons which can be disassociated by 0.35 M KCl. P30 can bind to the lighter complex to form a complex of greater than 300,000 daltons but does not bind to the 170,000 dalton complex. The proteins have a marked effect on the fidelity of transcription. The error rate of DNA transcription by purified RT is high, but in the presence of P30 or P12 the error rate is markedly reduced. In the presence of both P30 and P12 this error rate is reduced even further. Further studies suggest that only certain subclasses of P30 and P12 will so modulate the activity of RT. P30 can exist as a monomer or dimer. The dimer P30 appears to be much more active in binding to RT and altering its activity. Similarly P12 exists as phosphorylated and unphosphorylated proteins which may modulate the activity of RT to differing degrees.

An analogous picture of RT-gag protein complex emerges from an investigation of the virions themselves suggesting that the results of the in vitro study have biological significance.

Complexes of RT activity of approximately 600,000 and 200,000 daltons were routinely isolated from Triton lysed virus. The heavier complex is unstable with time and break down to form the 200,000 dalton complex. These complexes contain both P30 and P12, seemingly in equal molar ratios, but no GP70 or RNA. Functionally the complexes synthesize DNA with much fewer errors than does purified RT. The 200,000 dalton complex can be disassociated by salt to form 120,000 RT-P12 gag complex and a lighter 90,000 dalton complex. The RT-P12 complex displays an error rate in DNA synthesis greater than the 200,000 complex but less than the 90,000 unit whose rate approaches that of purified RT.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09129-01 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Nucleases as Differentiation Markers		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	G. Brown	Staff Fellow
		LMB, NCI
Other:	T. Karpetsky	Sr. Investigator
	R. Gallagher	Sr. Investigator
	R. Leavitt	Sr. Investigator
		LMB, NCI LMB, NCI COB, NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<u>Differentiation</u> of human <u>HL60</u> cells (established from a patient with <u>acute progranulocytic leukemia</u>) and mouse <u>peritoneal macrophages</u> is being induced by a variety of agents. Changes in the differentiation status of these cells is associated with changes in both the overall amount of <u>nuclease</u> activity and the numbers of activities being expressed. The changes in the macrophages are being followed in normal mice and in mice that have <u>genetic defects</u> in their immune system. The effect of the lymphokine <u>interferon</u> upon differentiation and nuclease expression is also being investigated.		

Mouse peritoneal macrophages exhibit nuclease activities similar to those expressed by peripheral blood leukocytes. However, nuclease activities increase quantitatively and qualitatively upon peritoneal injection of substances known to induce elicitation or activation of macrophages. Mouse strains are available that have genetic defects in macrophage function. The exact sites of these defects are unknown.

HL60 cells exhibit many of the properties of the leukemia cells from which they were derived. These cells exhibit DNase activities not found in cells from normal donors. These extra activities resemble those found in some populations of immature cells obtained from leukemia patients.

This study is designed to test the hypothesis that nuclease activities may be markers of differentiation. HL60 cells and mouse peritoneal macrophages are being stimulated to differentiate by a variety of agents. In some cases the differentiation process is being perturbed by interferon. The intracellular nucleases activities will be correlated with the state of differentiation and with the treatment.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09130-01 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) RNA Polymerase Type II and Other DNA Binding Proteins		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	G. Brown	Staff Fellow LMB, NCI
Other:	N. Biswal C.C. Levy	Sr. Investigator Chief LMB, NCI LMB, NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.25	0.25
CHECK APPROPRIATE BOX(ES)		
☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Competitive interactions between various oligodeoxyribonucleotides and calf thymus DNA-dependent RNA polymerase type II</u> are being characterized. Specificity of the enzyme for particular sequences has been demonstrated and found to reside in single stranded structures. <u>DNA binding proteins</u> from non infected cells and from cells infected by <u>Herpes simplex virus</u> (a known oncogenic virus) are being isolated and tested for their ability to preferentially bind to specific regions of HSV DNA. The effect of these proteins on the interaction of RNA polymerase with DNA is being evaluated.		

Herpes simplex viruses exhibit sequential genome transcription into at least 3 temporal classes. The mechanisms responsible for this sequential expression have not been elucidated but presumably involve either modifications of the RNA polymerase or induction of new DNA binding proteins.

This study is designed to help elucidate the nature of the interactions between RNA polymerase type II and DNA by investigating the ability of different single and double stranded oligonucleotides to competitively bind to RNA polymerase type II.

This allows a very sensitive determination of interactions, the development of a single comparison standard among many DNAs and the need to label only one DNA. Binding is measured by following the passage of the complexes through Sepadex G100 and by Millipore filter binding assays.

We are isolating double stranded DNA binding proteins and determining whether they preferentially bind to specific sequences of naked double-stranded HSV-DNA and how they affect binding and transcription of RNA polymerase type II.

We find that specificity resides not with any particular sequence on naked double-stranded DNA but only with single stranded DNA and can be manifested by regions as short as 2 bases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09131-01 LMB	
PERIOD COVERED October 1, 1979 to September 30, 1980			
TITLE OF PROJECT (80 characters or less) Chicken Liver Ribonuclease Distinguishes Cytidylic From Uridylic Acid Residues			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	C.C. Levy	Chief	LMB, NCI
Other:	T. Karpetsky	Sr. Investigator	LMB, NCI
COOPERATING UNITS (if any) None			
LAB/BRANCH Laboratory of Molecular Biology			
SECTION			
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201			
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	
0.4	0.3	0.1	
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) A heat-stable <u>endoribonuclease</u> isolated from <u>chicken liver</u> has been purified to homogeneity as evidenced by the presence of a single protein band upon polyacrylamide gel electrophoresis. The enzyme can, in limit digests of 5 S rRNA and 5.8 S rRNA, distinguish between cytidylic and uridylic acids bonds at a ratio of 6:1 and, therefore, may be useful in <u>RNA sequence analysis</u>. The means by which the enzyme hydrolyzes substrate is unusual in that kinetic data do not support a simple formation and breakdown of an enzyme-substrate complex. Rather, the existence of a second complex, consisting of 2 mol of substrate and one of enzyme, derived from the initial enzyme-substrate complex, is postulated. In common with the other endonucleases, enzyme activity is inhibited by free <u>poly(A)</u> or tracts of the polypurine present at the 3'-terminus of RNA. Reversal of inhibition and restoration of activity may be achieved by the addition of low concentrations of <u>spermidine</u> to reaction mixtures.			

At present, sequence analysis of RNA strands is dependent on a number of residue-specific enzymes. These RNases when used in concert with one another provide, through selective hydrolysis at specific bases, the means by which the sequential arrangement of nucleotides within a polymer chain can be pieced together. Although the most widely used sequencing reagent, bovine pancreatic RNase, will attack at pyrimidine bases, it does not distinguish between cytidylic and uridylic acids. The isolation from culture media of the slime mold, Physarum polycephalum, of an endonuclease (RNase Phys I) to which cytidylic acid bonds appeared resistant has to some extent mitigated the problem, although there may be a certain degree of ambiguity introduced into the identification of any nucleotide based solely on its activities. One means of getting around this difficulty is to have available a complementary enzyme, one which shows preferential activity toward cytidylic acid esters. Use of the two in tandem could give unequivocal identification of a pyrimidine nucleotide. The result of the work of this project was the isolation of just such an RNase from chicken liver.

By means of acid extraction, Sephacryl gel filtration, CM-cellulose chromatography, and CM Sephadex chromatography, the enzyme was purified to homogeneity (MW = 17,000). The chicken liver enzyme was found to discriminate between the two pyrimidines in RNA, the rate of attack on cytidylic acid containing internucleotide bonds being about 61 times as fast as that on uridylic acid bonds. Although there was also attack at adenylic acid sites as well, the preponderance of hydrolytic activity was again directed at cytidylic acid bonds, at a rate of 14:1 over that of adenylic acid residues. This proclivity of the enzyme for cytidylic acid residues suggests that the RNase when used in conjunction with other RNases as described above, should be useful in RNA sequence analysis.

Another characteristic of the enzyme which was also of interest was its interaction with polyadenylic acid (poly(A)). This polypurine, free or covalently linked to the 3'-termini of an RNA substrate, is an efficient inhibitor of enzyme activity. As has been demonstrated for the polypurine-induced inhibition of many endonucleases, spermidine, in low concentrations, can reverse the inhibition and restore enzyme activity.

This enzyme is currently in use in our laboratory to determine the nucleotide sequence at the 3' end of Rauscher leukemia virus. Also samples of the enzyme have been sent to investigators at many institutions world-wide to aid them in their sequence analysis problems.

Publications

Levy, C.C. and Karpetsky, T.P.: The purification and properties of chicken liver RNase. J. Biol. Chem. 255: 2153-2159, 1980

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09132-01 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Defective Virions of Moloney Murine Sarcoma-leukemic Viruses		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: N. Biswal Other: K. Baksi A. Meric C. Levy	Sr. Investigator Staff Fellow Jr. Investigator Chief	LMB, NCI LMB, NCI LMB, NCI LMB, NCI
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.6	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The broad aim of this project is to isolate and characterize defective deletion mutants of murine sarcoma-leukemia viruses. <u>Cloned infectious viruses</u> have been serially propagated in <u>tissue culture</u> and the extent of expression of the integrated viral DNA as extracellular viral specific <u>RNA</u> is now being examined by molecular hybridization techniques. Full length viral specific circular DNA was isolated from cells infected de novo with <u>virions</u> and cleaved with <u>restriction endonucleases</u> . These DNA fragments were electrophoretically separated on agarose gels, transferred and fixed on to nitrocellulose membrane filters and are now being examined for complementarity with radioactive viral RNA and c-DNA prepared with virions isolated at different passage levels. Those virion populations containing defective genome will be isolated and their role on the replication and integration of the DNA of infectious virions will be studied.		

Moloney sarcoma-leukemia viruses were cloned and serially propagated in Wistar rat embryo fibroblasts. At different passage levels the titers of the plaque forming leukemia viruses and focus forming sarcoma viruses were determined. Virus specific circular DNAs from cells infected denovo with the virions have been isolated. Experiments are in progress to cleave the circular DNAs with restriction endonucleases and hybridize the DNA fragments by "Southern" blot technique with RNA isolated from purified virions at different passage levels. Deletion mutants will be identified by correlating the nucleic acid data with the biological properties of the virions isolated at different passage levels.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09133-01 LMB
PERIOD COVERED October 1, 1979 to September 30, 1980		
TITLE OF PROJECT (80 characters or less) Role of Herpes Simplex Viruses in Malignant Transformation of cells		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	N. Biswal	Sr. Investigator
		LMB, NCI
Other:	K. Baksi G. Brown C. Levy D. Ross S. Akman	Staff Fellow Staff Fellow Chief Clinical Associate Clinical Associate
		LMB, NCI LMB, NCI LMB, NCI CO, NCI CO, NCI
COOPERATING UNITS (if any) Clinical Oncology Branch		
LAB/BRANCH Laboratory of Molecular Biology		
SECTION:		
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.2	0.9
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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER		
☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
Herpesviruses have been implicated in transformation of normal cells to malignant ones, and <u>viral specific macromolecules</u> (DNA, mRNA and proteins) have been detected in many tissues from <u>patients with carcinomas and lymphomas</u>. The purpose of this project is to (1) study the nature and physical state of the <u>viral DNA sequences</u> in malignantly transformed cells and human lymphoblastoid cells; (2) <u>clone the viral DNA sequence(s)</u> responsible for the maintenance of the state of cell transformation and (3) study the role of <u>DNA polymerase and DNA-binding proteins</u> in the viral DNA replication in the transformed cells. A small piece of DNA ($\sim 3 \times 10^6$ daltons) common to both HSV-1 and HSV-2 has been detected in some lymphoblastoid cells from patients with leukemia. Experiments are now in progress to clone and produce significant amounts of this DNA in <u>E. coli</u>. If successful, we plan to characterize the types of mRNA and proteins this viral DNA can specify in vitro and then use these RNA and protein products to detect viral specific information in lymphoblastoid or other related tissues from patients with leukemia and carcinoma.		

This project has three interrelated parts. The nature and physical state of viral DNA sequences are being investigated in three different types of cells. As a positive control and a model system, hamster cells transformed by herpes simplex virus type 2 (HSV-2) were used and we have detected a 5.4×10^6 dalton DNA fragment of this viral genome within the nucleus of these cells. Current efforts are directed to analyze the types of proteins and mRNA this viral DNA fragment specifies both in vivo and in vitro. The second type of cells chosen were transformed human lymphoblastoid cell lines K562 and HL60. K562 cells contain an additional thymidine kinase (TK) activity suspected (but not proven) to be very similar to that specified by herpesviruses. Direct analysis of the DNA of these cells for herpes simplex virus TK gene would resolve if the TK activity within these cells are viral specific or something else. Several experiments are in progress to resolve this question.

The third type of cells used in these experiments are lymphoblastoid cells from patients with various types of leukemia and lymphomas. DNA from these cells are being analyzed for sequence homology with DNA from various herpesviruses. A DNA fragment common to HSV-1 and HSV-2 have been detected in certain lymphoblastoid cells. To further characterize this DNA piece attempts are now being made to obtain large quantities of this DNA fragment by cloning in *E. coli* after ligation with plasmid DNA as vector. This is the second part of our studies.

The third part deals with the role of HSV-induced DNA polymerases and DNA-binding proteins in the viral DNA replication. Thus far, we have isolated and purified the DNA polymerases induced by either HSV-1 or HSV-2 through sequential chromatography on DEAE-cellulose, phosphocellulose and DNA-cellulose. We have found that DNA polymers rich in guanine plus cytosine were preferred over those rich in adenine and thymine and "activated" DNA polymers were preferred over intact DNA duplexes. While circular SV-40 DNA could not be copied, the polymerase demonstrated intrinsic capacity to copy native DNAs of HSV-1 and HSV-2 with almost equal efficiency. Such DNA products, synthesized after a 20 min. reaction by HSV-induced DNA polymerases with native HSV-DNA as template, hybridized with all the homologous HSV-1 DNA fragments generated by restriction endonucleases. An exonuclease, which digested [5- ^{32} P] phosphate labeled terminus of a double stranded DNA much more rapidly than the rest of the DNA chain, was found to be associated with both the HSV-induced DNA polymerase. This exonucleolytic activity can however be separated from the DNA polymerase activity by centrifugation in glycerol gradients. The role of this exonuclease on the fidelity of the DNA polymerase is now being investigated.

Publications

Biswal, N., Brown, G. and Levy, C. Characterization of the DNA polymerase of Herpes simplex virus type 1. *Biochemistry* (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 09134-01 LMB	
PERIOD COVERED October 1, 1979 to September 30, 1980			
TITLE OF PROJECT (80 characters or less) Ribonuclease Activity of Preparations of Human Lymphoblastoid Interferon			
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT			
PI:	T. Karpetsky	Sr. Investigator	LMB, NCI
Other:	C. Levy G. Brown N. Biswal A. Bandyopadhyay	Chief Staff Fellow Sr. Investigator Sr. Investigator	LMB, NCI LMB, NCI LMB, NCI LMB, NCI
COOPERATING UNITS (if any) Baltimore City Hospitals, Baltimore, Md. 21224; Dept. of Virology, Wellcome Research Laboratories, Kent, England			
LAB/BRANCH Laboratory of Molecular Biology			
SECTION			
INSTITUTE AND LOCATION NCI, NIH, Baltimore, Maryland 21201			
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.7	OTHER: 0.2	
CHECK APPROPRIATE BOX(ES)			
☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER			
☐ (a1) MINORS ☐ (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords) Crude human <u>lymphoblastoid interferon (IF)</u> has less <u>ribonuclease</u> RNase activity than equivalent primary <u>leukocyte IF</u> . RNase activity was abolished when the IF was purified by methods that differed from those that had failed to eliminate similar activity from leukocyte IF. It is thought unlikely that exogenous RNase plays a major role in the antiviral action of IF preparations. Basic <u>ribonuclease</u> activity, rather than simply the presence of protein could be detected using <u>polynucleotide-polyacrylamide gel</u> <u>electrophoresis</u> . Polyacrylamide gels were prepared containing low concentrations of a <u>polynucleotide</u> substrate. <u>Spermine</u> , present in high amounts during electrophoresis, enables the basic RNases to migrate in the presence of polyanionic substrates. Electrophoresis of the RNases and incubation of the gel at neutral pH followed by staining, results in a colored gel containing clear bands which define regions of enzyme activity. Higher resolution of activity regions was achieved by using polyacrylamide gel electrophoresis in one direction, followed by polynucleotide-polyacrylamide gel electrophoresis in a direction perpendicular to the first migration. Enzyme activities are visualized as colorless spots on a purple slab. Preliminary results also indicate that pretreatment leukemia patients have an additional lymphocyte nuclease activity when compared to lymphocyte activity patterns obtained from normal volunteers.			

It has been previously reported that partially purified human fibroblast interferon (IF) and partially and highly purified human leukocyte IF possess considerable ribonuclease (RNase) activity. Even a 300-fold purification of leukocyte IF (yielding a specific activity of 3.4×10^6 units/mg protein) resulted only in a 3-fold reduction (per mg protein) in the concentration of RNase. The relatively high levels of RNase activity associated with such purified IF add considerable complexity to the interpretation of experimental results.

Lymphoblastoid cells of the Namalwa line are another source of human IF, which is predominantly of the leukocyte-antigenic type. We have examined Namalwa IF to see if it contains RNase activity comparable to that of leukocyte IF. The purification steps of lymphoblastoid IF include precipitation with trichloroacetic acid, acid ethanol extraction and fractionation of the extracts, and antibody affinity chromatography.

RNase assays were performed on crude moderately and highly purified IF samples. Leukocyte IF, after purification by precipitation with acid thiocyanate, extraction with acid ethanol and fractionation of the extracts, still showed considerable RNase activity. For instance, even the most highly purified sample (purified about 300 fold, specific activity 3.4×10^6 units/mg protein), had 322 or 94 RNase units per mg protein or per 10^6 IF units respectively.

Lymphoblastoid IF was purified by different procedures and even a relatively moderate (50 fold) purification by TCA precipitation, ethanol extraction and fractionation resulted in a preparation free from measurable RNase activity. IF that was more highly purified (2000-2900 fold; specific activities $3.8-6.7 \times 10^6$ units/mg protein) by methods including affinity chromatography, was also devoid of RNase activity. The quantitation of individual enzyme activities, present in mixtures of proteins, is often complicated by the fact that two or more enzymes may have similar specificities. Thus, while it is possible to measure the total rate of substrate turnover, this figure can be misleading in that it may be the sum of several rates induced by different enzymes. The separation of enzymes may be accomplished using polyacrylamide gel electrophoresis and particular classes of resolved enzymes may be visualized. In order to do this, gels are cast containing a polynucleotide, and electrophoresis is conducted using a region of pH wherein the enzymes are inactive. Later, after incubation of the gel, the position of the enzyme may be visualized by subsequent staining.

Good results were obtained with this technique using purified enzymes, such as RNase A or any of the three bovine skeletal muscle ribonucleases. However, crude material, such as sonicated lymphocytes gave a streak on polynucleotide-polyacrylamide gel electrophoresis. Although streaking may result from the interaction between substrate and enzyme during migration, multiple activities with similar R_f s will also give similar results. To settle this question, and provide a means for high resolution of nuclease activities samples are first electrophoresed in a disc gel in the absence of polynucleotide substrate. Thin slab gels containing polynucleotide are then cast on the side of the discs and electrophoresis is accomplished in a direction perpendicular to that first used. After incubation and staining, activities are revealed as colorless spots on a purple field. The streaks of activity obtained with lymphocyte samples (10^6 cells) from pre-treatment acute myelogenous leukemia patients were

resolved into two spots of activity, one of which is not present in normal individuals. Studies are underway to confirm these observations using larger populations of individuals. Variation in activity patterns with change in tumor load will also be investigated.

Publications:

Karpetsky, T.P., Winchurch, R.A., Burman, C.J. and Fantes, K.H.: Ribonuclease activity of preparations of human lymphoblastoid interferon. *J. Gen. Virol.* 44: 241-244, 1979.

Karpetsky, T.P., Winchurch, R.A., Burman, C.J., and Fantes, K.H.: Ribonuclease content of preparations of lymphoblastoid interferon. *Interferon Sci. Me.* 7-8, 1978.

Davies, G.E., Karpetsky, T.P. and Levy, C.C.: The ribonucleases of bovine skeletal muscle. *Biochem. J.*: in press, 1980

Karpetsky, T.P., Davies, G.E., Shriver, K.K. and Levy, C.C.: Use of polynucleotide-polyacrylamide-gel electrophoresis as a sensitive technique for the detection and comparison of ribonuclease activities. *Biochem. J.*: in press, 1980

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